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Original article

The potential role of adjunctive ascorbic acid in the prevention of colistin-induced nephrotoxicity in critically ill patients: A retrospective study

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ABSTRACT

Background: Colistin is considered a valuable and last-resort therapeutic option for MDR gram-negative bacteria. Nephrotoxicity is the most clinically pertinent adverse effect of colistin. Vivo studies suggest that administering oxidative stress-reducing agents, such as ascorbic acid, is a promising strategy to overcome colistin-induced nephrotoxicity (CIN). However, limited clinical data explores the potential benefit of adjunctive ascorbic acid therapy for preventing CIN. Therefore, this study aims to assess the potential nephroprotective role of ascorbic acid as adjunctive therapy against CIN in critically ill patients.

Method: This was a retrospective cohort study at King Abdulaziz Medical City (KAMC) for all critically ill adult patients who received IV colistin. Eligible patients were classified into two groups based on the ascorbic acid use as concomitant therapy within three days of colistin initiation. The primary outcome was CIN odds after colistin initiation, while the secondary outcomes were 30-day mortality, in-hospital mortality, ICU, and hospital LOS. Propensity score (PS) matching was used (1:1 ratio) based on the patient's age, SOFA score, and serum creatinine.

Abbreviations: CIN, Colistin-induced Nephrotoxicity; MDR, Multiple drug resistance; XDR, Extensively drug-resistant; AKI, Acute Kidney Injury; PS, Propensity Score; AA, Ascorbic Acid; ICU, Intensive Care Unit; LOS, Length of Stay; CKD, Chronic kidney disease; HD, Hemodialysis.

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Results: A total of 451 patients were screened for eligibility; 90 patients were included after propensity score matching based on the selected criteria. The odds of developing CIN after colistin initiation were similar between patients who received ascorbic acid (AA) as adjunctive therapy compared to patients who did not (OR (95 %CI): 0.83 (0.33, 2.10), p-value = 0.68). In addition, the 30-day mortality, in-hospital mortality, ICU, and hospital LOS were similar between the two groups.
Conclusion: Adjunctive use of Ascorbic acid during colistin therapy was not associated with lower odds of CIN. Further studies with a larger sample size are required to confirm these findings.

1. Introduction

Nosocomial acquired infections due to multidrug-resistant bacteria (MDR) may be associated with increased mortality risk, especially among critically ill patients (Despotovic et al., 2020). Certain factors increase the risk of acquiring severe infections with MDR in the Intensive Care Unit (ICU) settings, such as prolonged use of broad-spectrum antibiotics (Prestinaci et al., 2015).

Recently, the clinical use of colistin has increased due to the high rate of bacterial resistance and limited development of new antibiotics with activity against gram-negative bacteria (Falagas et al., 2005). Colistin (known as Polymyxins E) is a bactericidal antibiotic in a concentration-dependent mode against gram-negative bacteria and indicated for life-threatening infections caused by MDR and extensively drug-resistant (XDR) bacteria (Falagas et al., 2005; Tsuji et al., 2019). It is considered the last-resort antibiotic due to its low therapeutic index and high risk of toxicity, particularly nephrotoxicity and neurotoxicity (Dai et al., 2019; Gai et al., 2019) Despite its reported high toxicity rates, it has relatively high clinical cure rates when given as a monotherapy or combination therapy. Recently, colistin became a vital option against MDR gram-negative species such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (Spapen et al., 2011).

The exact mechanism of colistin-induced nephrotoxicity (CIN) is not well-known (Gai et al., 2019; Temocin et al., 2015). However, it may be due to oxidative stress in the epithelium proximal tubules that leads to damage to the mitochondria (Gai et al., 2019). Colistin-induced nephrotoxicity is developed in approximately 60 % of the patients who received colistin (Javan et al., 2017). Many factors may be associated with the increased risk of developing CIN, such as underlying comorbidities, concomitant administration of nephrotoxic medications, presence of septic shock, administering high doses of colistin, and different colistin formulations (Pogue et al., 2011). Additionally, CIN might be a dose-limiting factor that either prevents administering the optimal therapeutic dose or leads to therapy interruption and increasing the risk of bacterial resistance (Dai et al., 2014; Sirijatuphat et al., 2015).

There is an evolving assumption about the coadministration of an antioxidant to protect the kidneys (Gai et al., 2019). Ascorbic acid is a water-soluble vitamin classified as a chain-breaking antioxidant and scavenger of free radicals (Padh, 1990). Many studies have evaluated the rule of ascorbic acid in critically ill patients (Shrestha et al., 2021). The results of studies on the benefit of ascorbic acid in acute kidney injury (AKI) are conflicting. A *meta*-analysis conducted earlier reported non-significant benefits of ascorbic acid in terms of mortality and a non-significant benefit in reducing the incidence of AKI in critically ill patients. However, it reduced the overall length of hospitalization. (Shrestha et al., 2021).

An early study was conducted on animals to assess the effect of ascorbic acid when co-administered with colistin and found that the coadministration of ascorbic acid resulted in a protective effect against tubular apoptosis and nephrotoxicity induced by colistin (Yousef et al., 2012). However, the clinical data exploring the potential benefit of ascorbic acid adjunctive therapy for preventing CIN is limited. Therefore, this study aims to assess the potential nephroprotective effect of ascorbic acid as an adjunctive therapy against CIN in critically ill patients.

2. Methods

2.1. Study design and setting

This retrospective cohort study included critically ill patients who received intravenous (IV) colistin during their ICU stay at King Abdulaziz Medical City (Riyadh), a tertiary-care academic referral hospital in Riyadh, Saudi Arabia. King Abdulaziz Medical city has several ICUs, including adult medical, surgical, trauma, and burn ICUs. The ICUs admit medical, surgical, trauma, and burn patients. The ICUs operate as closed units with 24/7 onsite coverage by critical care board-certified intensivists and clinical pharmacists. All the patients who met our inclusion criteria during the study period (01/01/2017 – 31/12/2020) were included. Patients were followed until they were discharged from the hospital or died during the in-hospital stay. The study was approved by Institutional Review Board (IRB) - King Abdullah International Medical Research Center (KAIMRC) in March 2021 (Ref.#NRC21R.062.02). Informed consent was not required; hence it has been waived off from the King Abdullah International Medical Research Center Institutional Review Board, Riyadh, Saudi Arabia, due to the retrospective observational nature of the study. All methods were performed following the relevant guidelines and regulations.

2.2. Study participants

We included adult (age $\geq$ 18 years) critically ill patients who received IV colistin therapy during their ICU stay. Patients were excluded if known to have Chronic kidney disease (CKD) on hemodialysis (HD), received a single dose of colistin, had AKI within 24 hours of ICU admission, no concomitant use of colistin and ascorbic acid, ICU LOS < 24 hours, or death within 24 hours of ICU admission (Fig. 1). Eligible patients were then classified into two groups based on ascorbic acid use as concomitant therapy within three days of colistin initiation.

2.3. Sample size calculation

A total of 84 patients (42 patients for each group) were required to achieve 80 % power to detect a nephrotoxicity differences between the group of prevalence 26 %. The prevalence in the control group who received colistin alone was assumed to be 54 % (Javan et al., 2017; Yousef et al., 2012). We expected that patients who received colistin in combination with ascorbic acid would have a reduction in the prevalence of nephrotoxicity by 28 %. The statistic test used is one-sided Z-Test with unpooled variance. The significance level of the test is 0.05.

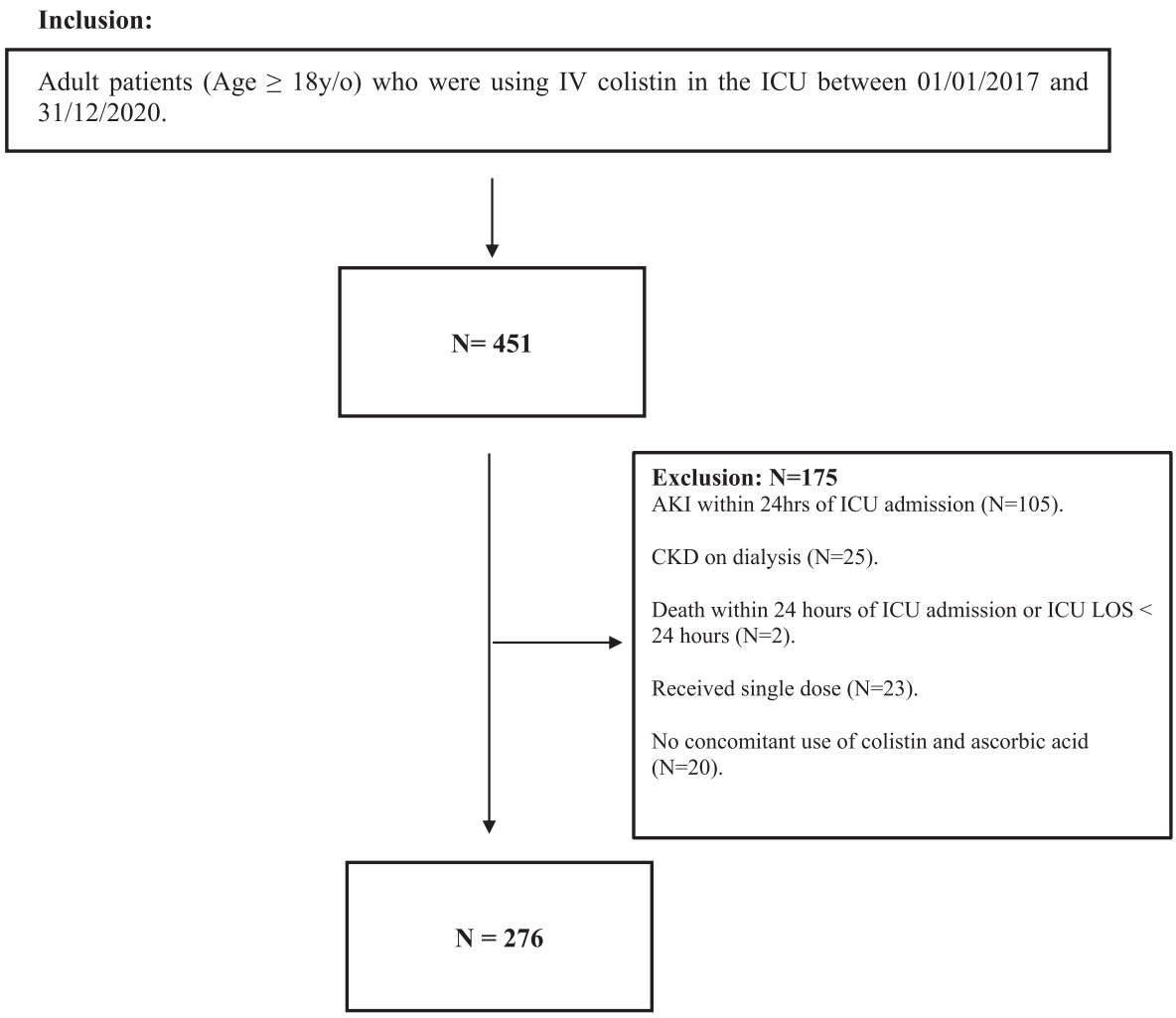

Fig. 1. Flow diagram of inclusion/exclusion criteria, and for eligible patients who underwent analysis.

2.4. Data collection

Each patients' data was collected and retrieved from the hospital system (BestCare). We collected patients' demographic data, comorbidities, vital signs and severity scores (Sequential Organ Failure Assessment Score (SOFA) and Acute Physiology and Chronic Health Evaluation II (APACHE II)), and mechanical ventilation status within 24 hours of ICU admission. Moreover, laboratory tests such as liver and renal profile, complete blood count, and lactic acid were collected. Furthermore, we record the following variables: timing of ascorbic acid and colistin initiation, duration of ascorbic acid and colistin therapy, and concomitant use of other nephrotoxic medications (i.e., vancomycin, aminoglycosides, and contrast). All patients were followed during ICU stay until they were discharged from the hospital or died during the in-hospital stay.

2.5. Outcomes

This study aims to assess the potential nephroprotective role of ascorbic acid as adjunctive therapy against CIN in critically ill patients. The primary outcome was CIN odds after colistin initiation. Secondary outcomes were 30-day mortality, in-hospital mortality, ICU, and hospital LOS.

2.6. Outcome definition (s)

- The 30-day mortality was defined as a death occurring for any cause within 30 days of the ICU admission date during a hospital stay; patients who were discharged from the hospital were presumed to be survived.
- CIN was defined as a sudden decrease of renal function, i.e., within 24-48 hours after colistin initiation, defined by an increase in absolute serum creatinine (SCr) of at least 26.5 μmol/L (0.3 mg/dL) or by a percentage increase in SCr ≥ 50 % (1.5 × baseline value) during ICU stay.(Mehta et al., 2007)

2.7. Statistical analysis

As appropriate, we presented numerical variables (continuous variables) as mean with standard deviation (SD), or median with lower and upper quartile (Q1 and Q3), and the categorical variables as number (percentage). The normality assumptions for all numerical variables were evaluated using a statistical test (the Shapiro–Wilk test) and graphical representation (i.e., histograms and Q-Q plots).

The two study groups' baseline characteristics and outcome variables were compared. We used the Chi-square or Fisher's exact

test for categorical variables as appropriate. The student *t*-test was used to compare normally distributed variables and the Mann-Whitney *U* test to compare non-normally distributed continuous variables. For the outcomes considered in this study, multivariable logistic, cox proportional hazards, and negative binomial regression analysis were used, and the results were reported as odds ratios (OR), hazard ratios (HR), or estimates with 95 % confidence intervals (CI) as appropriate. The regression analysis used the PS score as one of the model's covariates. No imputation was made for missing data, as the cohort of patients in our study was not derived from random selection. All statistical analyses were performed using SAS version 9.4, and a P-value of < 0.05 was considered statistically significant.

Based on the patient's age, SOFA score, and serum creatinine within 24 hours of ICU admission, the Propensity Score Matching Procedure (Proc PS match) (SAS, Cary, NC) was used to match patients who did not receive ascorbic acid with patients who did receive ascorbic acid as concomitant use with colistin. A greedy nearest neighbor matching method was used, with one patient without ascorbic acid (active) group matched with one patient who received ascorbic acid (control), resulting in the smallest within-pair difference among all available pairs treated patients. The difference in the logits of the propensity scores for pairs of patients from the two groups was matched only if it was less than or equal to 0.5 times the pooled estimate of the standard deviation.

3. Results

3.1. Demographic and clinical characteristics

Initially, a total of 451 patients were screened for eligibility, of which 276 patients were included. Patients who were administered colistin-only were 231 patients with a mean age of 54.4 years (SD ± 20.50), compared to 45 patients who administered colistin concomitant with ascorbic acid therapy (mean age: 47.2 years (SD ± 16.80). Most patients included in both groups were male (69.9 %). The most prevalent underlying comorbidities in both groups before PS matching were diabetes mellitus (41.7 %), followed by hypertension (40.9 %), dyslipidemia (13 %), and stroke (12.7 %) (Table 1).

There were notable differences between the two groups before propensity score matching; patients who received colistin-only were older, had a higher SOFA score, blood urea nitrogen (BUN), International Normalized Ration (INR), and mechanically ventilation (MV) needs within 24 h of ICU admission. Conversely, patients who received colistin concomitant with ascorbic acid therapy have a slightly higher median platelets count and albumin levels baseline.

Following the PS matching (1:1 ratio) based on the selected criteria, 90 patients were included. Most of the baseline characteristics and comorbidities were balanced between the two groups apart from mean body weight and body mass index (BMI), which were significantly higher in patients who received colistin concomitant with ascorbic acid therapy. There was no statistically significant difference between the two groups in concomitant nephrotoxic agent use or exposure (Table 1). The median cumulative dose of IV colistin as CBA in patients who received concomitant ascorbic acid was 68 mg/kg compared with 56 mg/kg in the other group. The median ascorbic acid dosage was 1000 mg enterally, with a median duration of 36 days.

3.2. Outcomes

3.2.1. Clistin-induced nephrotoxicity (CIN)

In both groups, older age and higher baseline serum creatinine levels were considered independent risk factors for CIN. In crude analysis after PS matching, CIN occurred in 16 patients (40 %) who received colistin-only compared to 16 patients (44.4 %) who received colistin concomitant with ascorbic acid therapy (p = 0.69). Moreover, in multivariable logistic regression analysis, there were no statistically significant differences between the two groups (OR 0.83 CI 0.33, 2.10; $p = 0.68$) (Table 2). The concomitant use of nephrotoxic medications was assessed and not statistically significant before and after PS matching between the two groups (Table 1).

3.3. Mortality & length of stay (LOS)

The 30-day mortality occurred in ten patients (22.2 %) who received colistin-only compared to 8 patients (17.8 %) who received colistin concomitant with ascorbic acid therapy (p = 0.59). In multivariable cox proportional hazards regression analyses, the 30-day mortality was higher in patients who received colistin-only; however, it did not reach statistical significance (HR 1.71 CI 0.65, 4.46; $p = 0.27$). Moreover, the in-hospital mortality was similar between the two groups (HR 1.19 CI 0.55, 2.58; $p = 0.66$) (Table 2).

Patients who received colistin concomitant with ascorbic acid therapy have a more prolonged ICU stay at crude analysis (35.0 days vs 20.0 days, p = 0.02). However, there were no statistically significant differences in ICU and hospital LOS between the two groups at regression analysis after using PS matching (Beta coefficient −0.05 CI − 0.43, 0.33; p = 0.81 and Beta coefficient 0.07 CI − 0.26, 0.41; p = 0.67 respectively) (Table 2).

4. Discussion

In this retrospective cohort study , patients who received colistin with ascorbic acid as adjunctive therapy had similar odds of developing CIN compared to patients who received colistin alone.In critically ill patients receiving colistin, we evaluated the effect of a low adjunctive dose of ascorbic acid. It has been demonstrated in preclinical studies that ascorbic acid has a protective effect against nephrotoxicity and tubular apoptosis caused by colistin (Yousef et al., 2012; Zavascki and Nation, 2017). These studies revealed that ascorbic acid could reduce CIN due to its antioxidant properties (Aslan et al., 2021; Yousef et al., 2012). The main mechanisms of CIN are acute tubular necrosis, manifested as decreased creatinine clearance (Ordooei Javan et al., 2015; Zavascki and Nation, 2017), and interstitial nephritis (Hatem et al., 2005). Moreover, CIN may be attributable to oxidative damage and inflammation (Ozyilmaz et al., 2011).

After propensity score matching, no significant difference in the odds of CIN between the two groups was observed in this study. These results are consistent with a randomized clinical trial conducted on 28 patients; the trial did not show any nephroprotection in the ascorbic acid group (Sirijatuphat et al., 2015). However, the nephroprotective effects of ascorbic acid could not be observed due to the small number of included patients in our analysis and the utilization of a lower dose than in other trials (2–4 g of ascorbic acid daily) (Dalfino et al., 2015; Kellum et al., 2012). Higher ascorbic acid dosages or longer administration times may have produced different results.

A prospective cohort study of critically ill patients with sepsis (Dalfino et al., 2015); had comparable results with preclinical studies (Aslan et al., 2021; Yousef et al., 2012), which showed that ascorbic acid was an independent protective factor against AKI in patients treated with colistin (Dalfino et al., 2015). In several trials, the incidence rate of CIN ranged from 0 to 53.5%, which is consistent with the findings of our study. In addition, the mean age in our

Table 1
Baseline characteristics.

	Before propensity score (PS) matching				After propensity score (PS) matching			
	Overall (N = 276)	**Colistin (N = 231)**	**Colistin + Ascorbic Acid (N = 45)**	**P-value**	**Overall (N = 90)**	**Colistin (N = 45)**	**Colistin + Ascorbic Acid (N = 45)**	**P-value**
Age (Years), Mean (SD)	53.2 (20.09)	54.4 (20.50)	47.2 (16.80)	0.0299^	45.5 (17.74)	43.8 (18.68)	47.2 (16.80)	0.2811^
Gender – Male, n (%)	193 (69.9)	161 (69.7)	32 (71.1)	0.8499^^	67 (74.4)	35 (77.8)	32 (71.1)	0.4684^^
Weight (kg), Mean (SD)	76.1 (20.90)	75.1 (20.95)	80.9 (20.19)	0.0875^	75.6 (22.29)	70.3 (23.24)	80.9 (20.19)	0.0230*
Body mass index (BMI), Mean (SD)	28.4 (7.79)	28.2 (7.61)	29.8 (8.63)	0.3817^	27.7 (8.18)	25.7 (7.25)	29.8 (8.63)	0.0388^
APACHE II score, Median (Q1, Q3)	17.0 (12.00, 26.00)	18.0 (12.00, 27.00)	12.0 (11.00, 23.50)	0.0869^	14.0 (10.00, 24.00)	15.0 (10.00, 24.00)	12.0 (11.00, 23.50)	0.9251^
SOFA score, Median (Q1, Q3)	7.0 (5.00, 9.00)	7.0 (5.00, 9.00)	6.0 (4.00, 7.00)	0.0096^	6.0 (4.00, 7.00)	6.0 (4.00, 7.00)	6.0 (4.00, 7.00)	0.5523*
Mechanical Ventilation, n (%)	214 (77.5)	188 (81.4)	26 (57.8)	0.0005**	3 (3.3)	1 (2.2)	2 (4.4)	0.2004**
PaO2/FiO2 ratio, Median (Q1, Q3)	199.8 (132.50, 324.00)	199.0 (132.50, 324.00)	233.0 (137.90, 341.40)	0.4252^	232.8 (135.30, 376.10)	223.9 (134.85, 383.60)	233.0 (137.90, 341.40)	0.7766^
Alanine transaminase (ALT) at admission (U/L), Median (Q1, Q3)	33.0 (19.00, 63.00)	33.0 (18.00, 68.00)	33.0 (21.00, 56.00)	0.7478^	30.5 (18.00, 56.00)	28.0 (17.00, 54.00)	33.0 (21.00, 56.00)	0.5914^
Aspartate transaminase (AST) at admission (U/L), Median (Q1, Q3)	41.0 (25.00, 70.00)	42.0 (25.00, 72.00)	37.0 (25.00, 55.00)	0.2809^	36.5 (22.00, 56.00)	35.0 (21.00, 66.00)	37.0 (25.00, 55.00)	0.7775^
Estimated glomerular filtration rate (eGFR) Baseline, Median (Q1,Q3)	96.0 (70.00, 130.00)	96.0 (70.00, 134.00)	93.0 (67.00, 124.00)	0.6034^	101.0 (65.00, 141.00)	109.0 (61.00, 154.00)	93.0 (67.00, 124.00)	0.1927*
Serum creatinine (umol/l), Median (Q1,Q3)	72.5 (58.00, 103.50)	72.0 (57.00, 101.00)	81.0 (62.00, 112.00)	0.2388^	75.0 (58.00, 108.00)	73.0 (57.00, 101.00)	81.0 (62.00, 112.00)	0.3682^
Urine output (UOP) (mls/kg/hour), Mean (SD)	1.1 (0.89)	1.1 (0.93)	1.1 (0.61)	0.4253^	0.9 (0.57, 1.55)	1.0 (0.53, 1.61)	0.9 (0.63, 1.53)	0.8941^
Blood urea nitrogen (BUN) at admission (mmol/l), Median (Q1,Q3)	6.9 (4.70, 11.75)	7.4 (5.00, 12.20)	5.0 (4.00, 6.90)	0.0006^	5.6 (4.00, 9.90)	6.4 (4.50, 11.90)	5.0 (4.00, 6.90)	0.0719^
Platelets count Baseline (10^9/L), Median (Q1, Q3)	250.0 (156.00, 337.00)	240.0 (149.00, 337.00)	268.0 (207.00, 335.00)	0.0851^	262.5 (167.00, 343.00)	241.0 (154.00, 346.00)	268.0 (207.00, 335.00)	0.1627^
Lactic acid baseline (mmol/l), Median (Q1, Q3)	1.6 (1.10, 2.60)	1.6 (1.09, 2.48)	1.7 (1.30, 2.80)	0.2962^	1.5 (1.10, 2.60)	1.5 (0.91, 2.18)	1.7 (1.30, 2.80)	0.0624^
Total WBC Baseline (10^9/L), Median (Q1, Q3)	11.7 (8.20, 15.40)	12.0 (8.50, 15.40)	11.1 (7.00, 15.40)	0.3404^	11.1 (7.40, 14.20)	11.0 (7.60, 14.00)	11.1 (7.00, 15.40)	0.9036^
International normalized ratio (INR), Median (Q1, Q3)	1.1 (1.07, 1.27)	1.2 (1.08, 1.28)	1.1 (1.00, 1.25)	0.0318^	1.1 (1.00, 1.25)	1.1 (1.08, 1.25)	1.1 (1.00, 1.25)	0.2838^
Activated partial thromboplastin time (aPTT) Baseline (Seconds), Median (Q1, Q3)	28.8 (25.90, 33.00)	28.9 (26.00, 33.00)	27.6 (25.10, 32.00)	0.1803^	28.3 (25.50, 32.00)	29.0 (26.10, 31.75)	27.6 (25.10, 32.00)	0.4236^
Total bilirubin (µmol/L), Median (Q1, Q3)	14.4 (8.30, 23.70)	14.6 (8.40, 25.90)	13.2 (7.80, 20.00)	0.1688^	13.8 (8.00, 21.60)	14.4 (9.60, 23.00)	13.2 (7.80, 20.00)	0.2867^
Albumin Baseline (gm/L), Median (Q1, Q3)	30.0 (27.00, 34.00)	30.0 (27.00, 34.00)	32.0 (28.00, 36.00)	0.0845^	32.0 (28.00, 36.00)	32.0 (28.00, 36.00)	32.0 (28.00, 36.00)	0.6391*
Blood sugar level (mmol/L) Baseline, Median (Q1, Q3)	8.7 (6.30, 12.70)	8.7 (6.30, 12.70)	8.7 (6.70, 12.60)	0.9306^	8.2 (6.30, 12.30)	8.1 (6.10, 11.60)	8.7 (6.70, 12.60)	0.5584^
Glasgow Coma Scale (GCS), **Median (Q1, Q3)**	7.0 (3.00, 13.00)	7.0 (3.00, 11.00)	9.0 (3.00, 15.00)	0.3100^	9.0 (3.00, 15.00)	8.5 (3.50, 15.00)	9.0 (3.00, 15.00)	0.9530^
Vasoactive Inotropic Score (VIS), Mean (SD)	31.2 (222.61)	34.0 (241.11)	16.5 (76.11)	0.1903^	10.3 (54.33)	4.0 (9.88)	16.5 (76.11)	0.9052^
Duration of Colistin (days) Median (Q1, Q3)	10.0 (4.00, 19.00)	10.0 (4.00, 19.00)	14.0 (5.00, 21.50)	0.1525^	11.0 (5.00, 21.00)	11.0 (4.00, 21.00)	14.0 (5.00, 21.50)	0.5718^
Concomitant use of Vancomycin use for two days or more, n (%)	208 (75.4)	172 (74.5)	36 (80.0)	0.4300^^	75 (83.3)	39 (86.7)	36 (80.0)	0.3961^^
Contrast use during ICU, n (%)	165 (59.8)	141 (61.0)	24 (53.3)	0.3348^^	52 (57.8)	28 (62.2)	24 (53.3)	0.3933^^
Concomitant use of Aminoglycosides (Amikacin OR Gentamicin) for two days or more	114 (41.3)	92 (39.8)	22 (48.9)	0.2587^^	40 (44.4)	18 (40.0)	22 (48.9)	0.3961^^
Comorbidity								
Chronic obstructive pulmonary disease (COPD), n (%)	16 (5.8)	15 (6.5)	1 (2.2)	0.2620**	3 (3.3)	2 (4.4)	1 (2.2)	0.5571**
Heart Failure, n (%)	16 (5.8)	16 (7.0)	0 (0.0)	0.0683**	1 (1.1)	1 (2.3)	0 (0.0)	0.3091**
Hypertension, n (%)	113 (40.9)	98 (42.4)	15 (33.3)	0.2565^^	25 (27.8)	10 (22.2)	15 (33.3)	0.2393^^
Diabetes mellitus, n (%)	115 (41.7)	97 (42.0)	18 (40.0)	0.8042^^	33 (36.7)	15 (33.3)	18 (40.0)	0.5117^^
Dyslipidemia, n (%)	36 (13.0)	28 (12.1)	8 (17.8)	0.3026^^	12 (13.3)	4 (8.9)	8 (17.8)	0.2148^^
Hypothyroidism, n (%)	18 (6.5)	16 (7.0)	2 (4.4)	0.5332**	3 (3.3)	1 (2.2)	2 (4.4)	0.5571**
Ischemic heart disease (IHD), n (%)	17 (6.2)	15 (6.5)	2 (4.4)	0.5967**	3 (3.3)	1 (2.2)	2 (4.4)	0.5571**
Chronic kidney disease (CKD), n (%)	14 (5.1)	12 (5.2)	2 (4.4)	0.8338**	4 (4.4)	2 (4.4)	2 (4.4)	>0.9999**
Venous Thromboembolism, n (%)	9 (3.3)	9 (3.9)	0 (0.0)	0.1782**	1 (1.1)	1 (2.2)	0 (0.0)	0.3146**
Liver disease (any type), n (%)	13 (4.7)	13 (5.6)	0 (0.0)	0.1031**	0 (0.0)	0 (0.0)	0 (0.0)	NA
Stroke, n (%)	35 (12.7)	32 (13.9)	3 (6.7)	0.1851^^	7 (7.8)	4 (8.9)	3 (6.7)	0.6939**

*T Test / ^ Wilcoxon rank sum test is used to calculate the P-value.
^^ Chi square/ ** Fisher's Exact teat is used to calculate P-value.

Table 2
Regression analysis for the outcomes after PS matching.

Outcomes	Number of outcomes/Total number of patients		P-value	Odds Ratio (OR) (95 %CI)	P-value $
	Colistin + Ascorbic Acid	Colistin			
Colistin-induced nephrotoxicity (CIN), n(%)Δ	16/36 (44.4)	16/40 (40.0)	0.69^^	0.83 (0.33, 2.10)	0.68
				Hazard ratio (95 %CI)	**P-value$***
30-day mortality, n(%)Δ	8/45 (17.8)	10/45 (22.2)	0.59^^	1.71 (0.65, 4.46)	0.27
In-hospital Mortality, n(%)Δ	12/45 (26.7)	14/45 (31.1)	0.64^^	1.19 (0.55, 2.58)	0.66
				beta coefficient (Estimates) (95 %CI)	**P-value $****
Hospital LOS, Median (Q1, Q3)Δ	56.0 (30.00, 105.00)	52.0 (26.00, 93.00)	0.71^	0.07 (−0.26, 0.41)	0.67
ICU LOS, Median (Q1, Q3)Δ	35.0 (16.00, 55.00)	20.0 (14.00, 31.00)	0.02^	−0.05 (−0.43, 0.33)	0.81

Δ Denominator of the percentage is the total number of patients.
^^ Chi-square/** Fisher test is used to calculate the P-value.
$ Multivariate logistic regression analysis is used to calculate Odds ratio and p-value.
$* Cox proportional hazards regression analysis is used to calculate hazard ratio (HR) and p-value.
$** Generalized linear model is used to calculate beta coefficient (estimates) and p-value.

study is relatively low (45.5 ± 17.74), and nephrotoxicity has proven to occur more significantly in patients older than 60 years of age (Balkan et al., 2014; Dalfino et al., 2015).

After propensity score matching, all patients included in our study had similar baseline kidney function. Two patients (4.4 %) in each group had chronic kidney disease. In our cohort, the concomitant use of vancomycin and aminoglycosides for two days or more were 83.3 % and 44.4 %, respectively. At the same time, the use of contrast media during ICU stay was 57.8 % (Table 1). Evaluating the effect of nephrotoxic agents such as; vancomycin, aminoglycosides, and contrast media administered during colistin treatment could affect the results. After propensity score matching, the baseline use of nephrotoxic agents was not significantly different between the groups in our study.

Among the 90 patients, we observed that the two groups had similar 30-day mortality, in-hospital mortality, and hospital LOS. In critically ill patients, ascorbic acid has been studied in multiple randomized and observational studies. A *meta*-analysis study was conducted to evaluate the effects of ascorbic acid administration on clinical outcomes. This *meta*-analysis included forty-four randomized trials, of which 16 trials were performed in an ICU settings. Ascorbic acid administration was not associated with a difference in mortality, AKI occurrance, ICU, and hospital length of stay compared with the control (Putzu et al., 2019).In addition, the current sepsis guidelines (Evans et al., 2021) recommend against ascorbic acid supplementation in critically ill patients. This recommendation was based on an updated analysis that included nine RCTs. Ascorbic acid did not reduce mortality compared to usual care (RR, 0.9; 95 % CI, 0.69–1.18). Further studies with higher quality may influence the future guidelines updates (Evans et al., 2021).

Our study has the advantage of describing a cohort of critically ill patients treated with colistin with or without ascorbic acid to assess its role in nephroprotection. However, it has several limitations; first, it was a retrospective observational design with a small sample size which may have influenced the study and hindered it from detecting group differences. Second, it was conducted at a single center, limiting its generability. Third the possibility of remaining confounders effect; in our study, the patient's age, SOFA score, and serum creatinine were controlled by statistical matching. Last, the lower dose of ascorbic acid than the doses utilized in other trials might limit reaching a renal protective effect; higher ascorbic acid dosages or longer administration times may have produced different results. Therefore, the results of this study could be used to support the need for further large-scale studies with controlling the confounders to determine whether ascorbic acid has a role in the prevention of CIN or not.

5. Conclusion

Adjunctive use of Ascorbic acid during colistin therapy was not associated with lower odds of CIN. Further studies with a larger sample size are required to confirm these findings.. Further studies with a larger sample size are required to confirm these findings.

Author contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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