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Randomised Controlled Trial

Evaluation of the effectiveness of N-acetylcysteine on accelerating the recovery of renal failure in patients with leptospirosis, a randomized clinical trial study

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ABSTRACT

Background: Limited studies have been conducted on patients with renal function recovery regarding severe leptospirosis. The purpose of this study is to evaluate the effectiveness of N-acetylcysteine (NAC) in accelerating the reduction of serum creatinine in patients with leptospirosis.

Patients and methods: This is a clinical trial study involving 64 patients with leptospirosis, with microscopic agglutination tests used to confirm the diagnosis of acute kidney injury. NAC was given to patients with a glomerular filtration rate of less than 60 ml/min at 1200 mg every 12 h, and it lasted for 48 h. Next, 32 patients were measured and the relationship between the length of hospitalization, age, and sex was also examined. Additionally, the two groups of case and control were compared in terms of the rate of decrease in serum creatinine level in three different time periods. The Shapro-Wilk test was used to investigate the distribution of data.

Results: No significant differences were observed in the decrease in serum creatinine level on the first, third, and seventh days of hospitalization and also in the use of NAC between the case and control groups ($P = 0.255$). In addition, the use of NAC had no significant effect on reducing the length of hospitalization ($P = 0.067$).

Conclusion: Recovery of acute kidney injury following leptospirosis and drugs that accelerate the healing process in these patients require further studies with greater sample size and longer follow-up time.

1. Introduction

Leptospirosis is a common disease among humans and animals. This disease occurs in both temperate and tropical regions. The incidence in the tropics is almost 10 times higher than that of the temperate regions [1,2]. Leptospirosis is a rare disease that is rarely reported, and there are no reliable statistics on its global incidence. Modeling by the World Health Organization's Leptospirosis Epidemiology Group (WHOLEG) estimates that there are 837,000 cases of this disease each year worldwide, with 48,600 deaths [3]. Different mammals are natural hosts, and humans are accidently infected after exposure either to animals or to the environment. In the United States, the incidence of leptospirosis is relatively low. Most cases have been reported from the southern coastal states and the Pacific. Accordingly, Hawaii consistently reports the greatest number of cases among other states. The prevalence of leptospirosis has also been reported among participants in Florida adventure races [4].

The clinical course of leptospirosis varies among different people. Most cases are mild and self-limiting or subclinical, while some others are severe and potentially fatal. The disease generally occurs in 75–100% of patients with a sudden onset of fever, myalgia, and headache after a period of 2–26 days (average of 10 days). Renal failure is often non-oliguric and associated with hypokalemia. In the acute phase,

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Abbreviations

NAC	N-acetylcysteine
GFR	Glomerular filtration rate
MAT	Microscopic agglutination test

conservative treatment may also be required in this regard. Of note, renal recovery is generally complete [5]. Urine analysis usually shows proteinuria, pyuria, granular cast, and sometimes microscopic hematuria [6]. Renal failure may be seen in severe leptospirosis. An increase in creatine kinase is mostly observed in approximately 50% of patients [7]. N-acetylcysteine (NAC) is an antioxidant that can regenerate glutathione. Furthermore, it was initially used to treat acetaminophen overdoses [8].

However, the use of NAC in the prevention of acute kidney injury (AKI) in various cases, such as contrast-induced AKI and postoperative, has been investigated with different results, mainly by measuring the change in serum creatinine level once before and once after NAC treatment. However, due to its low cost and lack of side effects, NAC has been recommended by Kidney Disease Improving Global Outcomes (KDIGO) for the use of acute contrast-induced kidney injury [9]. In addition, although NAC is generally recommended for patients with chronic kidney disease (CKD), supporting evidence is insufficient in clinical cases with a glomerular filtration rate (GFR) of less than 60 ml/min per 1.73^{m2} [9]. A recent systematic review by the Agency for Healthcare Research and Quality (AHRQ) supported the use of NAC to prevent AKI following contrast use [10].

Up to now, more than 20 experimental trials have investigated the effect of NAC on preventing AKI in various cases (AKI due to contrast agent, postoperative and result of medication) and also in CKD to reduce progression or to preserve residual renal function [11]. Due to the protective role of NAC, its antioxidant properties against harmful substances, its vasodilation effect in improving anoxia that can be used to prevent renal failure due to leptospirosis, and successful treatments in other cases of AKI and the lack of studies performed on its role in failure due to leptospirosis, we decided to investigate its effects on accelerating the recovery of renal failure in hospitalized leptospirosis patients to help in determining the appropriate strategy for these patients by recognizing the effects of NAC on improving renal failure in leptospirosis patients.

1.1. Patients and methods

This randomized clinical trial research was approved by the Mazandaran University of Medical Science Ethics Committee (approval number: IR.MAZUMS.REC.1398.1392 on 02- 26–2020) and was carried out in accordance with the Helsinki Declaration principles.

The authors confirm that all experiments were performed in accordance with the relevant guidelines and regulations. This research has been done with the conscious satisfaction of patients and all participants filled in a permission slip and was performed by block sampling and double-blind random sampling. The study population were patients with leptospirosis referred to Razi Hospital in Qaemshahr in 2020 who had reached a definitive diagnosis based on a microscopic agglutination test (MAT). According to GFR, the patients were divided into the following 4 groups: mild (60–89), mild to moderate (45–59), moderate to severe (30–44), and severe [15–20]. NAC was given to the participants with a GFR of less than 60 ml/min, 1200 mg every 12 h, and continued for up to 48 h. WBC, hemoglobin, platelets, sodium, potassium, BUN, creatinine, AST, ALT, and other related tests were also measured. Additionally, demographic information of the patients, including age, sex, job, length of hospitalization, duration of the improvement of clinical symptoms, and the mortality rate were recorded.

1.2. Data analysis

The minimum sample size required by considering the 95% confidence interval and 80% power of the study and using G power sample size determination software, 64 patients was determined, which in a one-to-one ratio can include 32 patients in the intervention group, and 32 in the control group. The Shapro-Wilk test and *t*-test were used to investigate the distribution of data. In the case of normal distribution of data, to describe the quantitative data, mean, standard deviation and median. Also, in the case of abnormal distribution, the median of the data, the maximum and the minimum will be presented. The statistical tests were used to investigate the relationship between NAC use and creatinine level reduction on the first, third, and seventh days of hospitalization and its relationship with variables such as length of hospitalization, age, and sex. The combined analysis was also used to compare the case and control groups in three time periods. Data description and analysis was performed using SPSS V.26 software and, in all cases, the value of P was less than 0.05 according to the statistical judgment criteria. The study will be double-blind, in which the patient and the final assessment will be blind to the type of study allocation.

2. Results

In this study, 32 patients with leptospirosis and renal failure received routine treatment (the control group) and the other 32 patients received NAC plus routine treatment (the case group) (Fig. 1). The creatinine level at the time of admission in the intervention group was 3.76 ± 0.76 on the first day after receiving routine care plus NAC. It was equal to 3.51 ± 0.73 on the third day, 2.67 ± 0.34 on the fourth day, and 2.31 ± 0.46 on the seventh day. The highest creatinine level in the intervention group after starting the treatment was 6.9 mg/dl on the first day and the lowest level on the seventh day was 1.8 mg/dl. The mean creatinine level at the time of admission in the control group was 3.53 ± 0.45. On the first day after receiving routine care, it was 3.33 ± 0.45, on the third day it was 2.67 ± 0.34, and on the seventh day it was 2.17 ± 0.33. Furthermore, the highest creatinine level in the control group after starting the treatment was 4.9 mg/dl on the first day and the lowest level was 1.6 mg/dl on the seventh day.

There was no significant difference between the group receiving NAC besides the routine treatments and the control group on the first, third, and seventh days, $P = 0.281$, $P = 000.1$, and $P = 0.264$, respectively. Most of the subjects in both the intervention and the control groups were men, 84.4% and 71.9%, respectively. There was no significant difference between the intervention and control groups in terms of the decreased serum creatinine level on the first, third, and seventh days after starting the treatment based on gender respectively ($P = 0.696$, $P = 0.915$, and $P = 0.691$). Notably, both sexes had the same distribution. The mean age of the participants in the intervention group was 37.88 to 8.86 years old and in the control group, it was 35.56 to 6.18 years old. Based on an independent *t*-test, there was no significant difference in age between the intervention and control groups ($P = 0.08$). Additionally, there was no significant difference in terms of length of hospitalization between those who received NAC (the intervention group) and the control group ($P = 0.06$). Based on repeated measure analysis of variance, the effect of NAC was not significant ($P = 0.255$), which means that serum creatinine levels in those individuals who received or did not receive NAC on the first, third, and seventh days were not significantly different.

Fig. 2 shows a gradual decrease in creatinine level over several days, which was similar in both groups, and there was no significant difference. Table 1 also shows the clinical signs of the participants.

3. Discussion

According to the results of this study, in the group of patients with leptospirosis who received NAC plus the routine treatments (intervention group), 84.4% of the participants were male patients with a mean

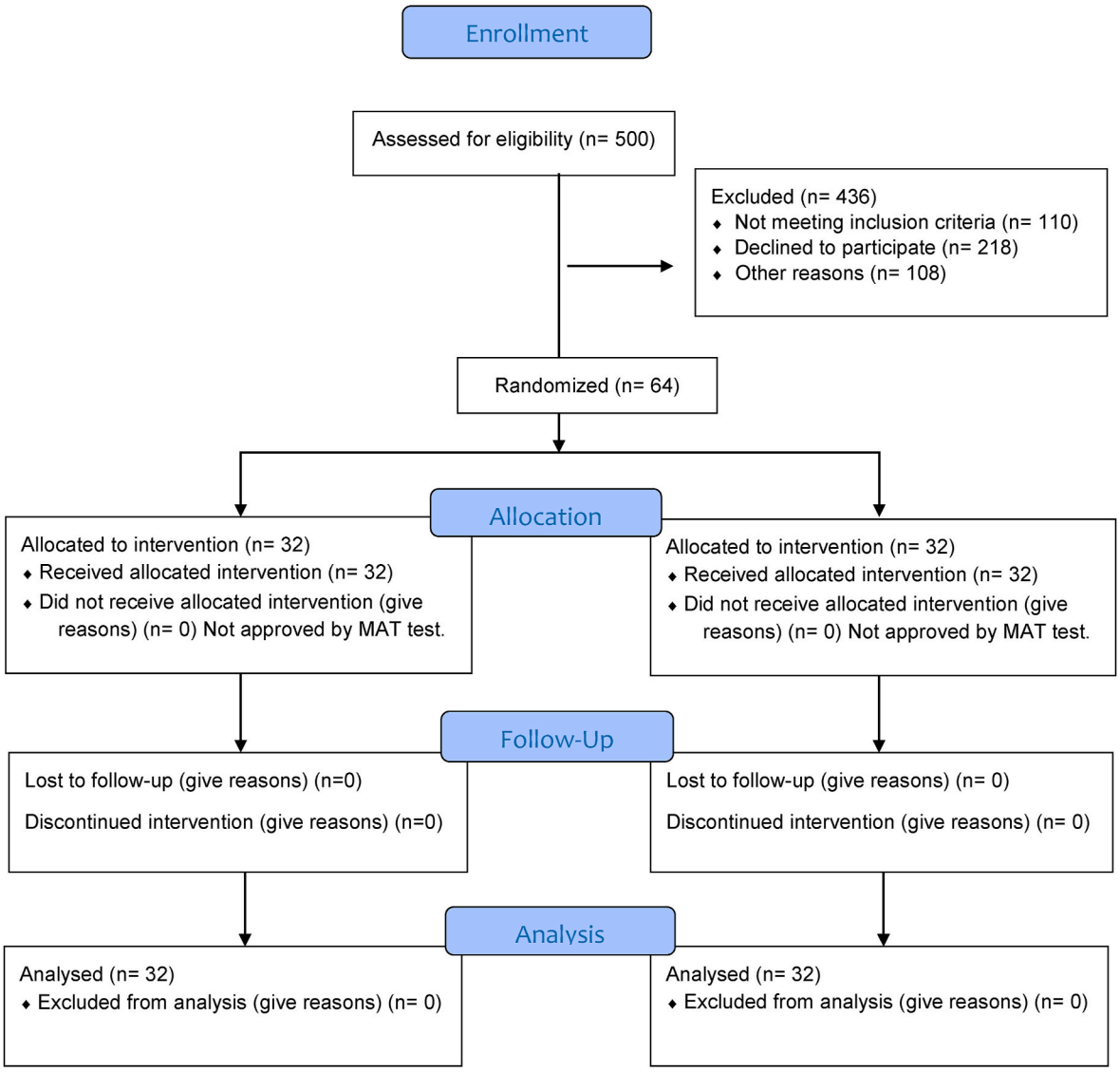

Fig. 1. The patient flowchart.

age of 37.8 ± 9.38 years old and 15.6% of them were women with an average age of 38 ± 6.04 years old. In the control group, 71.9% of the subjects were men with a mean age of 36.3 ± 6.03 years old and 28.1% of them were women with a mean age of 33.67 ± 6.51 years old.

The findings of this study indicated that there was no significant difference between the NAC receiving group and the control group, indicating that the data distribution was the same. Also, there was no significant difference in terms of length of hospitalization between those who received NAC (the intervention group) and the control group ($P = 0.067$).All of the patients had fever and myalgia.76.5% of the patients had headaches, 54.6% had conjunctivitis, 64.06% had meningitis, 28.1% had hemoptysis, and 3.1% of them had acute respiratory failure. In addition, thrombocytopenia was seen in 37.5% and arrhythmia in 4.6% of the subjects with atrial fibrillation. A change in the level of consciousness was observed only in one patient who also had acute respiratory failure.32.8% of patients had a WBC of less than 104 and 67.1% of them had more than 104/micl, 90.6% of the patients had hyponatremia, and 42.1% had elevated hepatic transaminases (ALT and AST). The main cause of oliguria in leptospirosis is dehydration [12–14]. 90.6% of the patients were dehydrated at the time of admission, but after suitable hydration, only 10.9% of the patients were found oliguric. At the time of admission, hyperkalemia was observed in only 3 patients (4.6%), while 56.2% of the patients had hypokalemia (Table 1). These observations indicate that AKI following leptospirosis is often hypokalemic and non-oliguric [13].

In addition to dehydration, jaundice and rhabdomyolysis are the other factors playing important roles in the pathogenesis and severity of AKI following leptospirosis [15,16]. Of note, all the patients in this study had jaundice. In addition to the direct function of Leptospira in the kidney, jaundice, rhabdomyolysis, and dehydration in leptospirosis were found as aggravating factors of AKI [16].

Despite the worsening of the disease, the clinical improvement of AKI following leptospirosis occurs in a relatively short time. In this study, only 8 patients (12.5%) needed more than 2 weeks of hospitalization. In a study by Cengiz et al. [17], it was stated that complete recovery in these patients was achieved between 3 and 5 weeks. However, when it is associated with acute respiratory failure or multiple organ failure, the mortality rate of the disease reaches 50% [18,19].

In the present study, only 1 patient (1.5%) died due to acute respiratory failure and pulmonary hemorrhage. Rapid clinical improvement and a low mortality rate, especially in areas endemic to leptospirosis, have been described in previous studies [13,20,21,and22]].In this study, 4 patients (12.5%) needed dialysis. Accordingly, this percentage is lower than the reported 23–52% in other studies [13,20,22]. Several

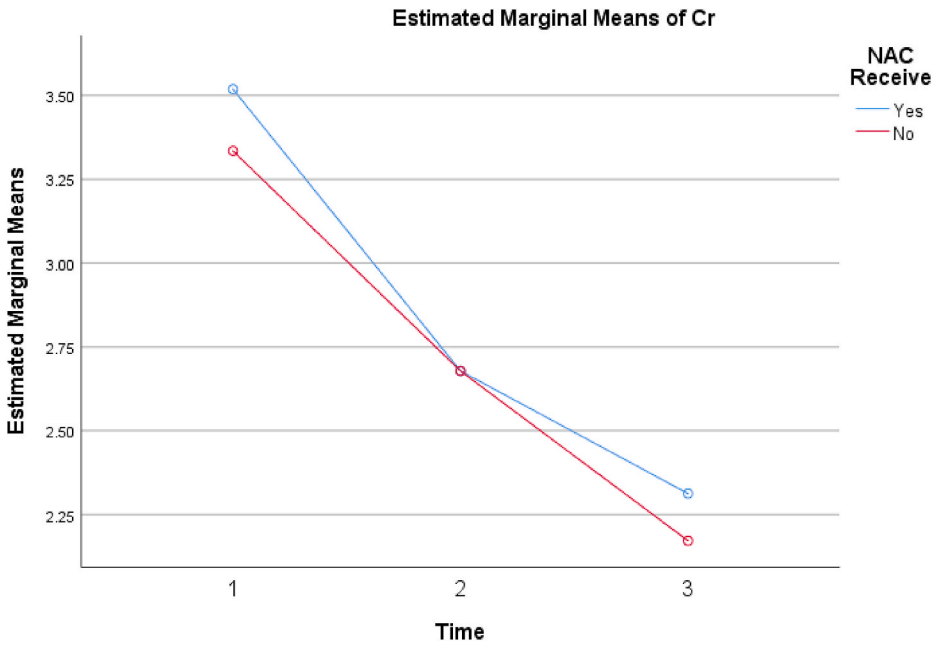

Fig. 2. The creatinine levels separately on different days.

Table 1
Clinical signs of the patients.

Clinical signs	Number (Percentage)	
	Control (n = 32)	Case (n = 32)
Fever	32 (%100)	32 (%100)
Headache	26 (%81.3)	23 (%71.9)
Myalgia	32 (%100)	32 (%100)
Conjunctivitis	15 (%46.9)	20 (%62.5)
Acute respiratory syndrome	–	2 (%6.3)
Skin rash	3 (%9.4)	4 (%12.5)
Meningitis	20 (%62.5)	21 (%65.6)
Jaundice	32 (%100)	32 (%100)
Hemoptysis	9 (%28.1)	9 (%28.1)
Thrombocytopenia	13 (%40.6)	11 (%34.4)
Acute renal failure	32 (%100)	32 (%100)
Dehydration	30 (%93.8)	28 (%87.5)
Oliguria after dehydration	4 (%12.5)	3 (%9.4)
Dialysis	–	4 (%12.5)
Mortality	–	1 (%3.1)
Arrhythmia	1 (%3.1)	2 (%6.3)
Mental status changes	–	1 (%3.1)

experimental studies have shown that tissue damage in the proximal tubules is earlier and more severe [23,24]. Functional studies in Indian pigs have reported that an indirect reduction is seen in proximal sodium uptake even in animals infected with normal GFR [24]. However, studies on human leptospirosis to evaluate proximal tubular dysfunction are still limited and debatable. Lin et al. [25], in a study on the tubular function of a patient in the recovery phase of AKI due to leptospirosis with normal GFR, but with increased urinary excretion of potassium followed by hypokalemia and metabolic alkalosis, observed no dysfunction of the proximal tubule (without bicarbonate, glucosuria, phosphaturia or uricosuria). Also, urinary excretion of potassium was attributed to the increased thickness of the ascending arch of the Henle.

However, Liamis et al. [26], in a study on a patient with leptospirosis with normal plasma clearance, reported proximal tubular dysfunction similar to Fanconi syndrome, along with phosphaturia, uricosuria, and renal glycosuria. In a study on 4 patients in the recovery phase, Yang et al. [27] reported AKI due to leptospirosis, proximal tubular defect in 1 patient, thick ascending Henle dysfunction in 2 patients, and normal tubular function in 1 patient. In a prospective analysis of 11 patients with acute leptospirosis-induced renal impairment, Serguro et al. [13] found that on the first and eighth days of hospitalization, sodium and potassium excretion and urinary potassium to sodium ratio increased. Also, the continuation decreased on the eighth day. Therefore, they concluded that due to the impaired proximal sodium reabsorption function in the acute phase, an increase occurs in sodium transfer in the distal region. In a study on 44 soldiers conducted by Simpson et al. after 1–14 years in the acute phase of leptospirosis, 11% of them had urinary incontinence [28]. In a study by Ooi et al. on 7 patients, urinary incontinence was seen in 2 patients who had been suffering for 6–22 months from AKI caused by leptospirosis [29]. When a septic or hemorrhagic shock is present and requires vasoactive drugs, the clinical course of AKI after leptospirosis is similar to that of acute tubular necrosis, with oliguria, hyperkalemia, and an increased mortality rate. Notably, renal function following renal tubular necrosis is impaired more than AKI due to leptospirosis [5]. One of the limitations of this study was the sample size of patients, so they have recommended performing future studies with a larger sample size. Another limitation of this study was the follow-up time of the patients, so it is recommended to increase the follow-up period of patients to every three months after discharge. Because in studies that investigated the pattern of improvement of AKI following leptospirosis, urinary incontinence was still observed for up to 6 months after the improvement of other parameters.

4. Conclusion

Recovery of AKI following leptospirosis and drugs that accelerate the healing process in these patients require further studies with a greater sample size and a longer follow-up time.

Ethics approval

The study was approved by the Ethics Committee of Mazandaran University of Medical Sciences (No: 1IR.MAZUMS.REC.1398.5593).

Authors' contributions

L.D and E.S designed the study, wrote the manuscript, and analyzed and interpreted the data. NRE, S.M, H.I and A.R, collected the data and provided critical comments. Z.Z involved in interpretation and editing

the manuscript. All authors read and approved the final version of the manuscript.

Consent for publication

Not applicable.

Registration of research studies

Trial registration; Iranian registry of Clinical Trials, Islamic Republic of Iran; IRCT registration number: IRCT20190727044343N4 on July 28, 2020. https://irct.ir/trial/41147.

Guarantor

Lotfollah Davoodi.

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Availability of data and materials

The authors are responsible for data. Access to all relevant raw data will be free to any scientist.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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Not applicable.

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