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Effects of adding hypertonic saline solutions and/or etilefrine to standard diuretics therapy in cirrhotic patients with ascites

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Abstract. – OBJECTIVE: The renin-angiotensin-aldosterone system (RAAS) activation is the milestone in ascites formation. Hypertonic saline solution (HSS) has attracted considerable interest over the last years in ascites control. Other therapeutic models and concepts have been introduced to overcome diuretic resistance and control ascites. We aimed to evaluate the effects of adding HSS infusion and/or etilefrine to oral diuretics therapy on inflammatory and metabolic pathways, renal and systemic hemodynamics, and clinical outcomes by estimating the changes in selected biochemical and biological markers in cirrhotic patients with ascites.

PATIENTS AND METHODS: Ninety cirrhotic patients with ascites were studied after administration of HSS infusion (n=25) or etilefrine tablets (n=25), or both (n=25) plus standard diuretics therapy (SDT), or SDT alone (n=15). Serum levels of interleukin-6 (IL-6), aldosterone, leptin, and C-reactive protein (CRP). Hepatic and renal functions were measured at baseline, after eight days, then after 38 days.

RESULTS: A significant reduction in serum IL-6, serum aldosterone, Child-Pugh score, MELD-Na score, and increase in serum leptin, and mean arterial pressure ($p<0.05$) were noted after 38 days in HSS and combination groups. A significant improvement in diuresis, in all groups, urinary sodium excretion, and creatinine clearance ($p<0.05$) were increased after 38 days in all groups except the SDT group.

CONCLUSIONS: The results suggest that HSS, etilefrine, and their combination plus SDT are superior to SDT alone for ascites control and can exert some benefits on clinical, systemic, inflammatory, renal, and metabolic pathways without renal or hepatic dysfunction.

Key Words:
Ascites, Serum IL-6, Plasma aldosterone, Serum leptin.

Abbreviations

AASLD: American Association for study of liver disease; ADHF: Acute decongestive heart failure; ANOVA: One-way analysis of variance; CBC: Complete blood count; Crcl: Creatinine clearance; CRP: C-reactive protein; GFR: Glomerular filtration rate; HSS: Hypertonic saline solution; IL-6: Interleukin-6; INR: International normalized ratio; LPS: Lipopolysaccharide; MAP: Mean arterial pressure; MELD-Na: Model for end-stage liver disease with sodium score; ODS: Osmotic demyelination syndrome; RAAS: Renin-angiotensin-aldosterone system; SDT: Standard diuretics therapy; SIRS: Systemic inflammatory response syndrome.

Introduction

Ascites is considered the dark side in the course of disease in liver cirrhosis due to portal hypertension and vasodilatation accompanied by poor prognosis and increased risk of mortality. Once ascites has developed, the prognosis worsens with a one-year mortality rate of 12% and a five-year mortality rate of 44%[1]. The most predictive factors of poor prognosis include hyponatremia, low MAP, low renal Na excretion, and glomerular filtration rate (GFR), which consider a big challenge in disease control. 5% to 10% of patients with compensated cirrhosis per year develop this complication[2].

Salt restriction and diuretics are recommended as first-line therapy in many clinical guidelines. However, salt restriction depends on experts' opinion, and some randomized controlled trials have suggested a lack of benefit with a sodium-restricted over an unrestricted diet, even an increase in ascites and renal complications have been reported[3]. The main reasons that put salt restriction under debate are it can increase the risk of protein-malnutrition due to impaired taste, loss

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of appetite, and diminished dietary intake with early satiety as a result of over fluid compression on the abdomen, which may result in sarcopenia and increase morbidity and mortality risk[4].

Adding vasoconstrictor drugs such as terlipressin and midodrine consider the second line in ascites management[5]. Midodrine, an α- adrenergic agonist, can be added to standard medical therapy to control ascites to improve systemic hemodynamics without renal and hepatic dysfunction[6].

Clonidine a centrally acting α2-adrenergic agonist, showed rapid mobilization of ascites by a significant decrease in sympathetic activity and renin-aldosterone levels[7]. Etilefrine is another sympathomimetic agent with potent α-adrenergic agonist and mild β1- and β2 adrenergic agonist activities. It has a potent vasoconstrictor effect in hypotensive circulatory disorders (Vascon® – Drug information pamphlet, https://hipharm-eg.com/product/vascon-dps-tab/).

HSS restores mean arterial pressure (MAP) by shifting water from interstitial compartments to vascular compartments by osmotic gradient shifting, which improves peripheral tissue perfusion and cardiac preload[8].

Translocation of microorganisms from the intestinal lumen to the mesenteric lymph nodes promotes the release of pro-inflammatory cytokines and increases nitric oxide production, which aggravates the existing vasodilation. Data showed an anti-inflammatory effect of HSS via decreased cytokines released by neutrophils in response to lipopolysaccharide (LPS)[9].

Leptin, the adipocyte-derived hormone, contributes to various biological functions through its role in energy metabolism and activation of the immune system. Leptin links immune function/hemostasis, metabolism, and nutritional status. Leptin deficiency is associated with dysregulation of cytokine production, which increases susceptibility to infection and inflammation. Therefore, leptin considers a profibrogenic factor and modulates the severity of liver disease in cirrhotic patients[10].

Trial Objectives

The primary objectives were to evaluate and compare the impact of such a treatment regimen on plasma aldosterone, serum IL-6, serum leptin, and serum CRP in cirrhotic patients with ascites. Furthermore, urine output and MAP were assessed.

Secondary objectives were to evaluate the change in ascites grades, liver disease scores (Child-Pugh score and MELD-Na score), hepatic and renal function, and CBC.

Other objectives were to assess the safety and tolerability of such treatment regimens.

Patients and Methods

Study Design

The study was a parallel, randomized, active-controlled, single-center study conducted at the Hepatology Department of the National Liver Institute (NLI) from November 2017 to April 2020.

Ethics

The study was approved by the Institution Review Board (IRB) of NLI, Menoufia University, Egypt, with NLI/IRB protocol number: 00131/2017, and the Research Ethics Committee of the Faculty of Medicine, Tanta University, approval code: 32624/10/18. The study's design and methods were consistent with the Helsinki Declaration and its subsequent amendments in 1964. Informed consent was obtained from all individual participants included in the study.

Study Population

All patients were recruited from the Hepatology Department of National Liver Institute, Menoufia University, from November 2017 to April 2020. Patients should be on diuretics therapy and did not undergo paracentesis before. All demographics, baseline characteristics, and clinical examinations were recorded. Patients were randomized into four groups: SDT group received oral standard diuretic therapy (SDT) furosemide 40 mg plus spironolactone 100 mg with dose increased in 40 mg: 100 mg ratio as needed, HSS group received 150 ml of HSS infusion (1.4%-3%) plus SDT, etilefrine group received oral etilefrine 5 mg 3 times daily plus SDT, and combination group received 150 ml of HSS infusion (1.4%-3%) and oral etilefrine 5 mg 3 times daily plus SDT.

Oral diuretics and etilefrine administration were continued from the start to the end of the study (38 days). Diuretics dosage was reassessed according to blood pressure, diuresis, and sodium and potassium levels. No albumin infusion nor antibiotics were received during the study period as a condition for completing the study.

HSS infusions were administered for eight days under hospital observation. The small volume of the HSS (150 ml) was determined based

on its dose of 2 ml/kg (average weight 70 kg) and its concentrations calculated according to serum sodium of patients and previous clinical studies.

For serum Na < 135 mmol/L, HSS concentration between 2.4% and 3% (410.7- 513.4 mmol/L NaCl).

For serum Na ≥ 135 mmol/L, HSS concentration at 1.4% (239.6 mmol/L NaCl).

Samples Collection and Outcome Assessment

Venous blood samples were drawn from enrolled patients in the morning before treatment (first measurement), after eight days (second measurement), then after 38 days (third measurement). Blood samples were centrifuged, and the resulting supernatant was frozen at -80°C until all samples were collected. 24-h urine was collected in the morning before treatment, after eight days, and after 38 days to assess diuresis and urinary creatinine, serum Na, and K.

Crcl was calculated from serum and 24-hour urinary creatinine [24-h urine creatinine concentration × 24-h urine volume]/[serum creatinine × 1,440 min/d].

Liver disease scores (Child-Pugh and MELD-Na scores) were assessed at the beginning, after eight days, then after 38 days.

Inclusion and Exclusion Criteria

All study participants were clinically evaluated before participating to confirm matching criteria and exclude all potential contraindications.

Inclusion Criteria

- All cirrhotic patients with ascites grade I-III;
- Patients ages from 25 -65 years;
- Patients were on diuretics and did not undergo paracentesis.

Exclusion Criteria

- Non-cirrhotic ascites.
- Active gastrointestinal bleeding.
- Congestive heart failure.
- Acute renal failure (or serum creatinine ≥ 2 mg/dl).
- Hepatocellular carcinoma.
- All Cancer types.
- Arterial hypertension or diabetes mellitus.
- Acute infection.

Randomization

The participants in the study who were eligible for inclusion were assigned numbers in the order (1, 2, 3, 4, 5) according to their admission first. The patients assigned numbers (1, 2, 3, and 4) were allocated to the three treatment groups (HSS group, Etilefrine group, and combination group), and patients assigned number 5 were allocated to the control groups who received SDT. Then the patients of the treatment groups were equally randomized into the three treatment groups according to their assigned numbers. This method was adopted to overcome the problem of patients withdrawing from treatment groups and performed by the hospital pharmacy at national liver institutes. The trial was open-label.

Statistical Analysis

Data were analyzed using IBM SPSS software package version 22.0 (IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to verify the normality of the distribution. Numbers and percentages for qualitative data. Mean ± SD for normally distributed quantitative data; otherwise, median with (IQR) was used. Chi-square test for categorical variables. Normal and abnormal distributed quantitative variables were compared between groups by one-way analysis of variance test (ANOVA) and Kruskal Wallis test with post-hoc test (Tukey) and (Dunn's) for pairwise comparisons between every two groups, respectively, while ANOVA with repeated measures test and Friedman test for intragroup analysis with pots-hoc test (Bonferroni adjusted) and (Dunn's) for pairwise comparison between periods, respectively. $p<0.05$ was taken as significant. The final changes (Δ) were evaluated by the difference between the values of the variables at baseline and after 38 days (baseline value-after 38 days).

Results

A total of 118 Cirrhotic patients with ascites grades I-III were evaluated for eligibility, and 105 patients were enrolled and randomized for reasons illustrated in a flow diagram (Figure 1). Twenty-eight patients were in the treatment groups, and 21 patients were in the control (SDT) group. Ninety patients (45 male /45 female) completed the 38 days of treatment.

Effect on the Clinical Characteristics

Baseline data revealed non-significant differences in age, weight, MAP, and cirrhosis etiology among groups, while there were significant differences in the patients' sex, Child-

Pugh score, and MELD-Na score (Table I).

Significant changes in body weights were observed among the four groups (p=0.002). It reduced significantly in HSS, etilefrine, and combination groups by (2.5±3.0 kg; p<0.001, 1.88±3.46 kg; p=0.013, 2.96±2.46 kg; p<0.001, respectively) and increased non-significantly in the SDT group (Figure 2A).

Final changes in MAP were significant between the four groups (p=0.003), data showed significant improvement in HSS, and combination groups by (-5.52±7.85 mmHg; p=0.001, and

Table I. Baseline characteristics of all patients.

Parameters	SDT group n = 15	HSS group n = 25	Etilefrine group n = 25	Combination group n = 25	p
Sex					†<0.001
Male, n (%)	6.0 (40)	18 (72)	4.0 (16)	17 (68)	
Female, n (%)	9.0 (60)	7.0 (28)	21 (84)	8.0 (32)	
Age (years)	56.13 ± 7.32	57.92 ± 5.92	54.36 ± 6.10	57.32 ± 5.47	‡ 0.185
Weight (kg)	90.20 ± 19.87	89.76 ± 18.60	77.88 ± 14.71	84.56 ± 15.85	‡0.060
Etiology, n (%)					
HCV	9.0 (60)	18 (72)	19 (76)	22 (88)	
Bilharzia	3.0 (20)	4.0 (16)	4.0 (16)	3.0 (12)	† 0.193
HCV + Bilharzia	3.0 (20)	2.0 (8.0)	0.0 (0.0)	0.0 (0.0)	
Unknown	0.0 (0.0)	1.0 (4.0)	2.0 (8.0)	0.0 (0.0)	
Child-Pugh score	8.07 ± 1.79	8.68 ± 1.49	7.36 ± 1.08	8.32 ± 1.31	‡0.010
MELD-Na score	13 (11-16)	20 (19-24)	14 (11-17)	19 (14-21)	§<0.001
MAP (mmHg)	84.33 ± 9.71	82.99 ± 7.64	79.59 ± 9.15	78.81 ± 6.13	‡ 0.092
BUN (mg/dl)	15 (9.5-18)	22.4 (16.8-28.2)	13.18 (11.2-16.82)	18.22 (12.33-30.9)	§0.005
Serum creatinine (mg/dl)	1.0 (1.0-1.0)	1.15 (0.95-1.44)	0.71 (0.58-0.84)	1.1 (0.88-1.41)	§<0.001
Urinary creatinine (mg/day)	72.0 (56.5-79.0)	60.0 (49.5-86.7)	62.74 (34.3-74.1)	60.0 (46.1-83.0)	§0.680
24-h urine output (ml/day)	950 (825-1,100)	1,060 (980-1,160)	920 (600-1,100)	1,020 (910-1,120)	§0.063
Crcl (ml/min)	62.0 (36.0-73.0)	33.18 (31.47-51.98)	49.42 (29.3-61.39)	41.12 (35.28-49.16)	§0.146
Urinary Na (mmol/day)	76.0 (60.0-110)	33.0 (21.6-54.0)	53.0 (43.7-71.1)	52.3 (43.7-65.0)	§ <0.001
Urinary K (mmol/day)	29.0 (22.0-31.0)	20.5 (14.69-36.0)	30.58 (21.1-45.0)	21.2 (17.1-26.4)	§0.033
Serum Na (mmol/L)	137.4 ± 3.92	128.37 ± 5.73	134.28 ± 4.34	129.88 ± 4.11	‡<0.001
Serum K (mmol/ L)	4.13 ± 0.52	3.9 ± 0.55	4.24 ± 0.56	3.98 ± 0.60	‡ 0.156
Serum albumin (gm/dl)	3.0 (3.0-4.0)	2.73 (2.3-3.3)	3.29 (3.02-3.89)	2.6 (2.3-3.0)	§ 0.001
Serum ALT (U/L)	38 (28.5-48.5)	25 (15.7-30.8)	27.2 (21.8-37.37)	25.2 (15-32)	§ 0.021
Total bilirubin (mg/dl)	1.0 (0.0-2.5)	1.45 (1.0-1.9)	1.37 (0.92-2.3)	1.57 (1.27-1.94)	§0.515
Prothrombin activity (%)	68.33 ± 12.31	61.52 ± 11.38	72.55 ± 13.93	64.58 ± 11.88	0.016
INR	1.0 (1.0-1.5)	1.33 (1.25-1.49)	1.28 (1.2-1.45)	1.34 (1.25-1.44)	§ 0.028
Hemoglobin (gm/dl)	11.4 ± 2.26	10.93 ± 1.67	10.88 ± 2.04	10.68 ± 1.6	‡0.707
WBCs (10^3/uL)	5.0 (3.0-5.0)	5.7 (4.5-7.1)	4.1 (3.2-4.9)	5.4 (4.1-6.5)	§ 0.009
Platelets (10^3/uL)	96 (84-144)	103 (71-149)	105 (76-134)	99 (80-128)	§ 0.964
Serum CRP (mg/L)	24.0 (14.5-43.5)	36.1 (23.0-55.8)	12.7 (11.8-7.5)	27.9 (20.7-41.5)	§0.009
Serum IL-6 (pg/ml)	41 (37.5-60.5)	51.6 (46.37-73.81)	50.49 (46.0-53.2)	67.82 (48.7-74.32)	§ 0.035
Plasma aldosterone (pg/ml)	84 (69-100)	103 (66-143.2)	79.72 (38.88-118.3)	86.26 (66.59-107.1)	§0.316
Serum leptin (pg/ml)	848 (253-1,201)	1,012 (260-1,733)	1,034 (360-1,518)	1,092 (677-1,294)	§ 0.771

Data expressed as n (%), (mean ± SD), or median (IQR). †, for a chi-square test; ‡, for ANOVA test; §, for Kruskal-Walis test. p, for comparison between the groups; Significance at p<0.05. MAP, mean arterial pressure; BUN, blood urea nitrogen; Crcl, creatinine clearance; MELD-Na, a model for end-stage liver disease depending on sodium; CRP, c-reactive protein; IL-6, interleukin-6.

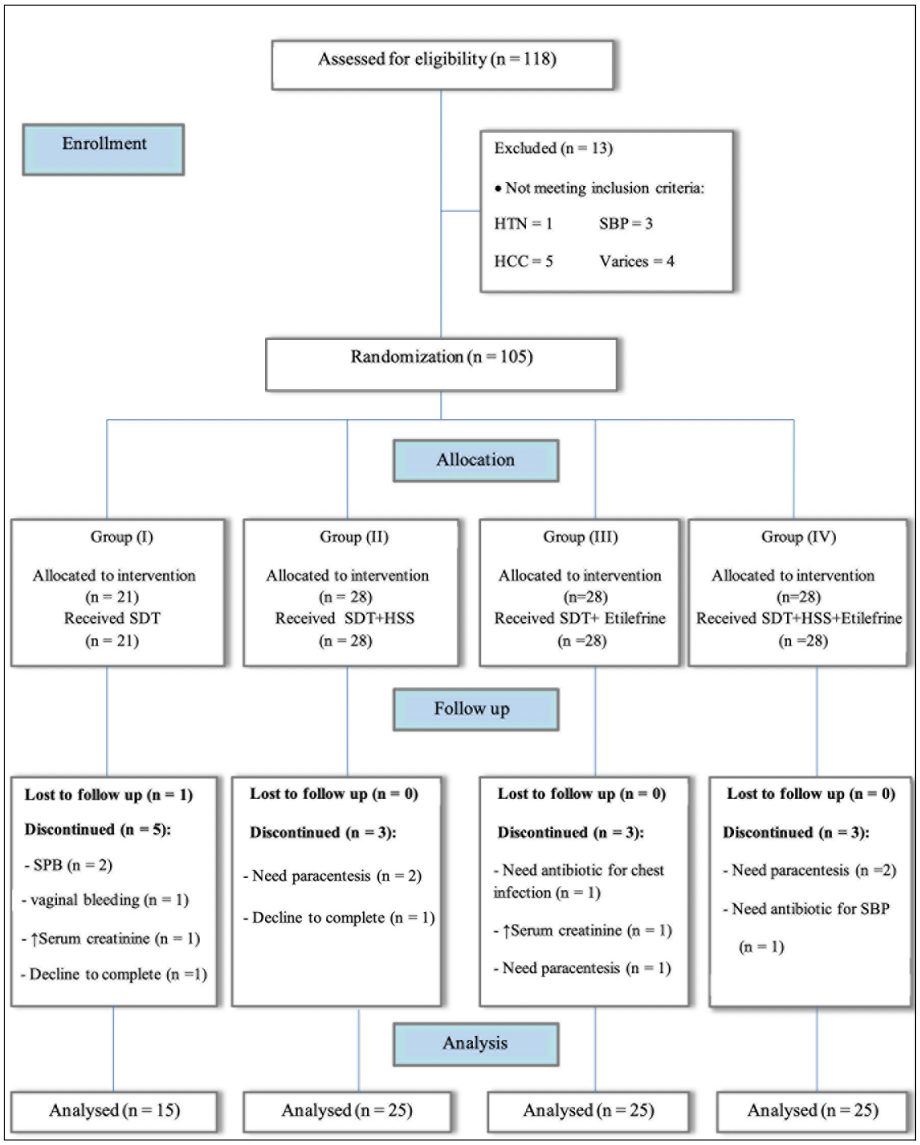

Figure 1. Flow diagram of participants. SBP: Spontaneous bacterial peritonitis, HTN: Hypertension, HCC: Hepatocellular carcinoma, SDT: Standard diuretic therapy, HSS: Hypertonic saline solution.

-7.04±6.71 mmHg; $p<0.001$, respectively) without significant change in SDT, or etilefrine groups (Figure 2B).

Significant changes in the MELD-Na scores were noted among the four groups ($p<0.001$), results showed significant MELD-Na reduction in HSS, and combination groups by [4.0 (0.0-8.0); $p<0.001$, 5.0 (2.0-7.0); $p<0.001$, respectively] without significant changes in the SDT or etilefrine groups (Figure 2C).

Effect on Renal Function

Final changes in median BUN were significant between four groups ($p=0.004$). While a significant increase was observed in the SDT group by [-4.0 (-7.5 - -2.5) mg/dl; $p<0.001$], no significant change can be noted in other groups. The final changes in median serum creatinine were non-significant ($p=0.166$) among the four groups, it reduced significantly in the combination group by [0.15 (0.05 - 0.25) mg/dl; $p=0.001$] without significant reduction in other groups. Urinary creatinine concentrations differ significantly between the four groups ($p<0.001$), with a significant increase in HSS and combination groups by [-6 (-12.30 - 2.50) and -4.3 (-9.83 - 1.10); $p=0.021$, similarly], and a significant reduction in the SDT and etilefrine groups by [23 (6.0 - 40.50); $p=0.008$ and 12 (-2.64 - 26.39); $p=0.009$, respectively].

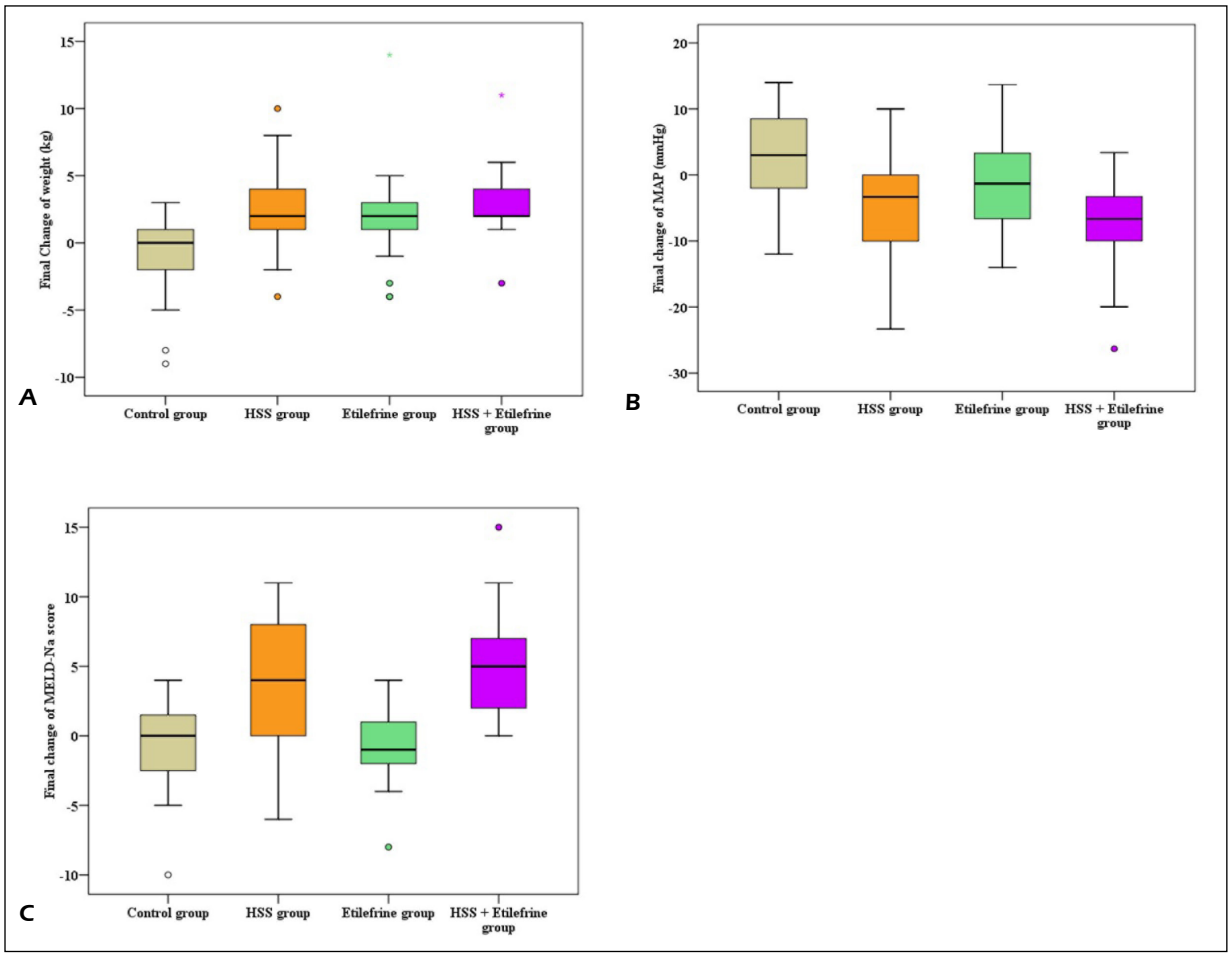

Figure 2. Effect of treatment regimens on the clinical characteristics. **A**, Body weight. **B**, MAP. **C**, MLD-Na score. The figure shows a non-significant increase in the body weight in the SDT group with a significant decrease in the other three groups, a significant increase in MAP in HSS and combination groups without significant change in SDT or etilefrine groups, and a significant decrease in MELD-Na score in HSS and combination groups without significant change in SDT or etilefrine groups. Final changes are calculated by the difference between baseline values – after 38 days values.

Diuresis improved significantly between the four groups (p=0.004) with significant increase in all groups by [-350 (-575-170) ml/day; p=0.002, -970 (-1050 - -550) ml/day; p<0.001, -600 (-950 - -300) ml/day; p<0.001, -750 (-910 - -510) ml/day; p<0.001, respectively] in favor of HSS group. Crcl showed significant changes among the groups (p<0.001) at the end of the study. It increased significantly in HSS, etilefrine, and combination groups (p<0.001), without significant improvement in the SDT group (Figure 3A,B).

Furthermore, significant change can be observed in urinary Na concentrations among the groups (p=0.001), with a significant increase in HSS, etilefrine, and combination groups (p<0.001, similarly), and a significant decrease in the SDT group (p=0.031). Also, significant changes were noted in serum Na among the four groups, with a significant increase in HSS and combination groups (p<0.001, similarly), by (-4.8±5.14 mmol/L, -4.22±2.20 mmol/L, respectively), and a significant decrease in SDT group by 3.20±4.75 mmol/L (p=0.010) without significant change in the etilefrine group (Figure 3C,D).

Effect on Hepatic Function

Serum albumin showed significant changes among the four groups (p<0.001), with a significant increase within HSS and combination groups (p=0.002 and p<0.001, respectively), without considerable changes in SDT or etilefrine groups. Serum ALT did not show significant changes among groups, while a significant reduction can be noted within the HSS group (p=0.019).

Effect on Blood Picture

Data showed non-significant changes in Hb, WBCs, and platelets within four groups at the end of the study (p=0.107, 0.103, 0.897, respectively)

Effect on a Panel of Biological Markers

As shown in (Table II, and Figure 4), the final changes in median CRP concentrations were significant between the four groups (p<0.001). Intragroup analysis showed significant reduction in HSS, etilefrine, and combination groups (p<0.001, similarly) by (21.8 (9.9 - 42.3) mg/L, 6.0 (4.0 - 12.0) mg/L, and 13 (7-20) mg/L), with significant increase in SDT group by [-1.0 (-7.5 - 3.0) mg/L; p=0.003].

Data revealed a significant change in serum IL-6 among all groups (p=0.005). Intragroup analysis showed significant reduction in HSS, and combination groups by (6.7 (-4.1-16.3) pg/ml; p=0.025, and 4.0 (1.0 - 12.0) pg/ml; p=0.007, respectively), the decrease in etilefrine group was non-significant (p=0.289), with non-significant increase in SDT group by -6.0 (-14.0 - 2.0) pg/ml, p<0.164.

Furthermore, the final changes in plasma aldosterone were significant among all groups (p=0.001). Intragroup analysis showed significant reduction in HSS and combination groups by (13.1 (3.5 - 42.58) pg/ml; p=0.003, and 16 (7.0 - 27.0) pg/ml; p<0.001, respectively), without significant changes in etilefrine or SDT groups (p=0.340, 0.091, respectively).

Moreover, serum leptin showed significant changes among all groups, while intragroup

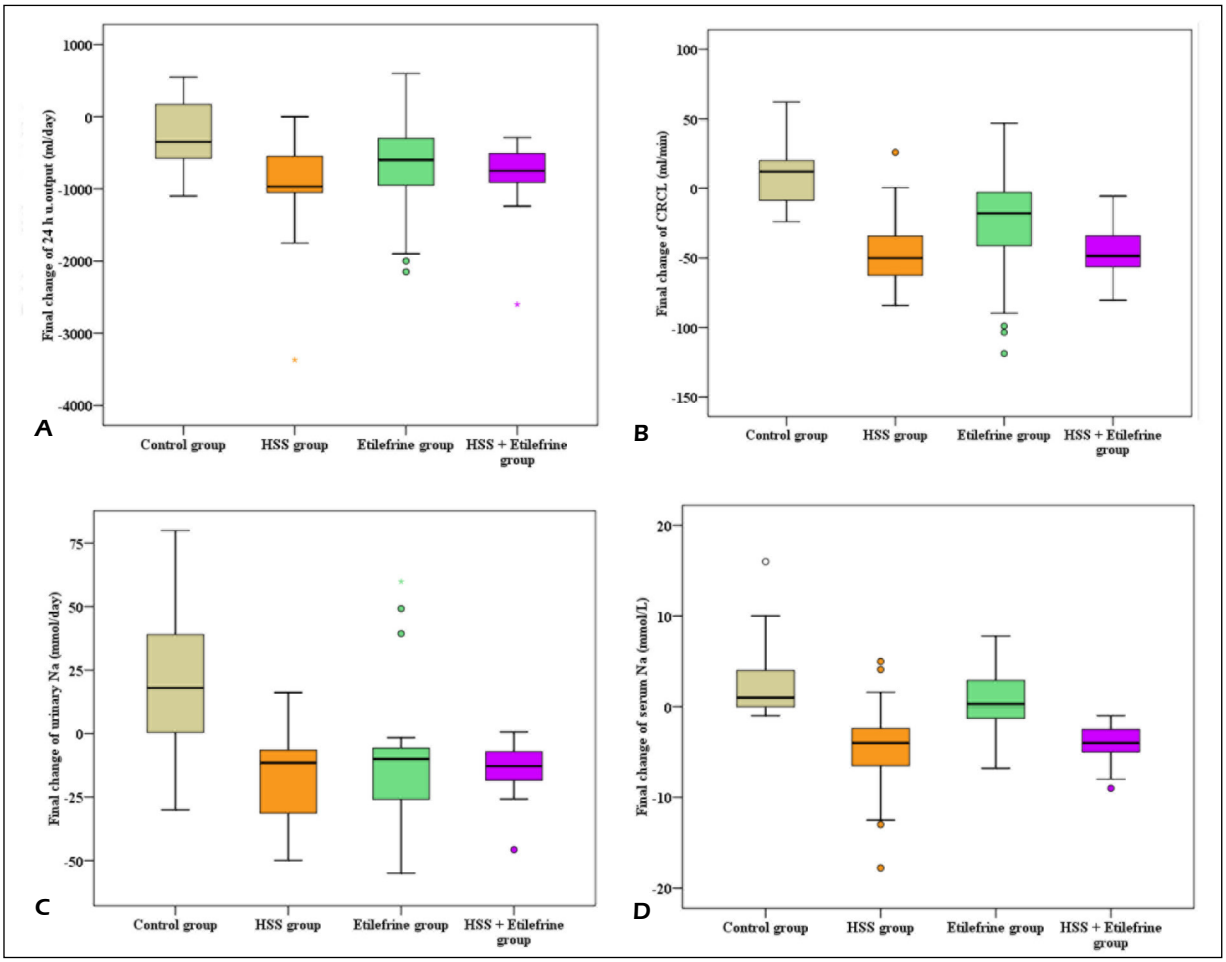

Figure 3. Effect of treatment regimens on renal function. **A**, Urine output. **B**, Crcl. **C**, Urinary Na concentration. **D**, Serum Na concentration. Urine output showed a significant improvement in all groups in favor of the HSS group, crcl showed significant improvement in the three treatment groups without significant change in the SDT group, urinary Na concentration increased in the three treatment groups with a significant decrease in the SDT group, and serum Na showed a significant increase in the HSS and combination groups and a significant decrease in the SDT group, without considerable change in the etilefrine group. Final changes are calculated by the difference between baseline values – after 38 days values.

Table II. Effect of the treatment regimens on the biological markers.

Parameters	SDT group n = 15	HSS group n = 25	Etilefrine group n = 25	Combination group n = 25	§p
Serum CRP (mg/L) At baseline After 8 days After 38 days	24.0 (14.5-43.5) 20.0 (13.0-31.5) 25.0 (14.0-43.5)	36.1 (23.0-55.8) 24.5 (9.1-32.5) 12.5 (8.3-15.6)	12.7 (11.8-27.5) 10.4 (8.3-20.2) 9.8 (6.5-12.9)	27.9 (20.7-41.5) 19.0 (14.5-31.5) 12.3 (8.6-22.3)	0.009 0.056 0.001
Serum IL-6 (pg/ml) At baseline After 8 days After 38 days	41.0 (37.5-60.5) 44.0 (35.5-58.5) 50.0 (35.5-70.5)	51.6 (46.37-73.81) 52.0 (43.0-65.0) 51.0 (45.0-60.0)	50.49 (46.06-53.20) 48.09 (45.22-53.87) 47.15 (43.82-50.03)	67.82 (48.70-74.32) 60.22 (43.65-71.48) 62.11 (39.82-68.91)	0.035 0.083 0.344
Plasma aldosterone (pg/ml) At baseline After 8 days After 38 days	84.0 (69.0-100) 93.0 (72.0-105.5) 129 (73.0-191)	103 (66-143.2) 82.24 (39.87-130.6) 65.0 (42.26-108)	79.72 (38.88-118.3) 79.81 (36.33-112.7) 66.34 (26.74-116.5)	86.26 (66.59-107.1) 80.11 (45.96-110.7) 66.23 (43.1-91.32)	0.316 0.792 0.031
Serum leptin (pg/ml) At baseline After 8 days After 38 days)	848 (253-1,201) 1,033 (289.5-1,245) 725 (346-1,281)	1,012 (260-1,733) 1,446 (1,132-1,780) 1,554 (862-1,984)	1,034 (360-1,518) 996 (790-1,980) 1,195 (506-2,801)	1,092 (677-1,294) 1,365 (964-1,756) 1,210 (963-2,034)	0.771 0.270 0.169

Data expressed as (mean± SD), or median (IQR); §, for Kruskal-Wallis' test. *p*, for comparing between the studied groups; Significance at $p<0.05$. CRP, C-reactive protein; IL-6, Interleukin-6.

comparison showed significant increase in HSS, and combination groups by (-436 (-1023 - -94.0) pg/ml; $p=0.004$, and -604 (-752 - -72.0); $p=0.002$, respectively), without significant increase in SDT or etilefrine groups ($p=0.085$, 0.595, respectively).

Post-Hoc (Tukey, Dunn's) analysis showed non-significant differences between HSS and combination groups ($p>0.05$) and the superiority of etilefrine on diuretics alone ($p<0.05$). Patients in HSS and combination groups had the highest weight reduction, MAP improvement, Child-Pugh score reduction, MELD-Na score improvement, urinary creatinine and urinary Na excretion, diuresis and Crcl improvement, serum Na elevation, serum albumin and serum leptin increase, serum CRP, serum IL-6, and plasma aldosterone reduction (Figure 2-4).

Safety and Tolerability Assessment

All treatment regimens were generally well-tolerated and safe. No episodes of gastric bleeding, HE, or HRS were reported. Minor mouth dryness was noted in one patient in the HSS group. Hypokalemia was observed in three patients in the HSS group due to increased urinary K excretion, while we did not report this side effect with the etilefrine group. No neurological manifestation of osmotic demyelination syndrome (ODS) was reported during HSS infusion as this condition was recorded with rapid serum Na correction in malnourished and hyponatremic patients, and we consider this. No vein extravasation was reported as this condition was associated with a high HSS concentration of over 3%, which needed a central vein catheter, and fewer concentrations were applied. No effect of HSS on coagulopathy was noted with our small, applied concentrations. No cases of acute hypotension were reported with HSS as all solutions were infused with a rate of 2 ml/min, and a rapid infusion rate accompanied acute hypotension. No report of hyperosmolar renal failure with HSS as reported with mannitol infusion. Our results suggest that hypertonic saline and etilefrine are safe, tolerable, and do not result in major adverse effects.

Discussion

Our study aimed to evaluate whether adding HSS, etilefrine, or their combination to SDT could provide some benefits to the inflammatory pathway (serum IL-6, serum CRP), metabolic pathway (serum leptin), renal hemodynamics, and functions (plasma aldosterone, 24 h urine output, Crcl, and urinary Na excretion,), and systemic hemodynamic (MAP) in cirrhotic patients with ascites.

The HSS has attracted considerable interest over the last years in treating cerebral edema, shock, lung extravasation, and congestive fail-

ure[11]. Several studies have demonstrated the efficacy of HSS infusion in regional blood flow insufficiency by restoring central hemodynamics and peripheral blood flow. HSS infusion produces rapid elevation of extracellular Na and osmotic pressure leading to plasma volume expansion and fluid mobilization from extracellular to vascular compartment. Also, HSS infusion augment diuretics action of furosemide to overcome diuretic resistance and high diuretics doses[12]. Furthermore, data showed that salt supplementation favors salt restriction in improving diuresis through increasing GFR in the treatment of patients with ADHF[13].

Our findings were consistent with those of previous studies concerning diuresis improvement as the highest diuresis in favor of the HSS group and the lowest diuresis for the SDT group. Despite the increase in urine output and urinary creatinine excretion in HSS, and combination groups, we did not record a significant decrease in serum creatinine concentration in the HSS group despite its significant decrease in the combination group, contrary to prior studies[13]. In both cases, it reinforces the results of previous studies on its role in maintaining kidney function by decreasing vascular resistance and enhancing renal blood flow without renal abnormalities[14].

The target in ascites control is Na balance achievement rather than strict Na restriction. A non- sodium-restricted diet can increase serum Na, urinary Na excretion, renal blood flow, and decrease levels of aldosterone and plasma renin[15]. Our results clearly showed enhancement of renal function *via* improvement of serum Na and natriuresis in HSS and combination groups.

Our results match those in previous studies that

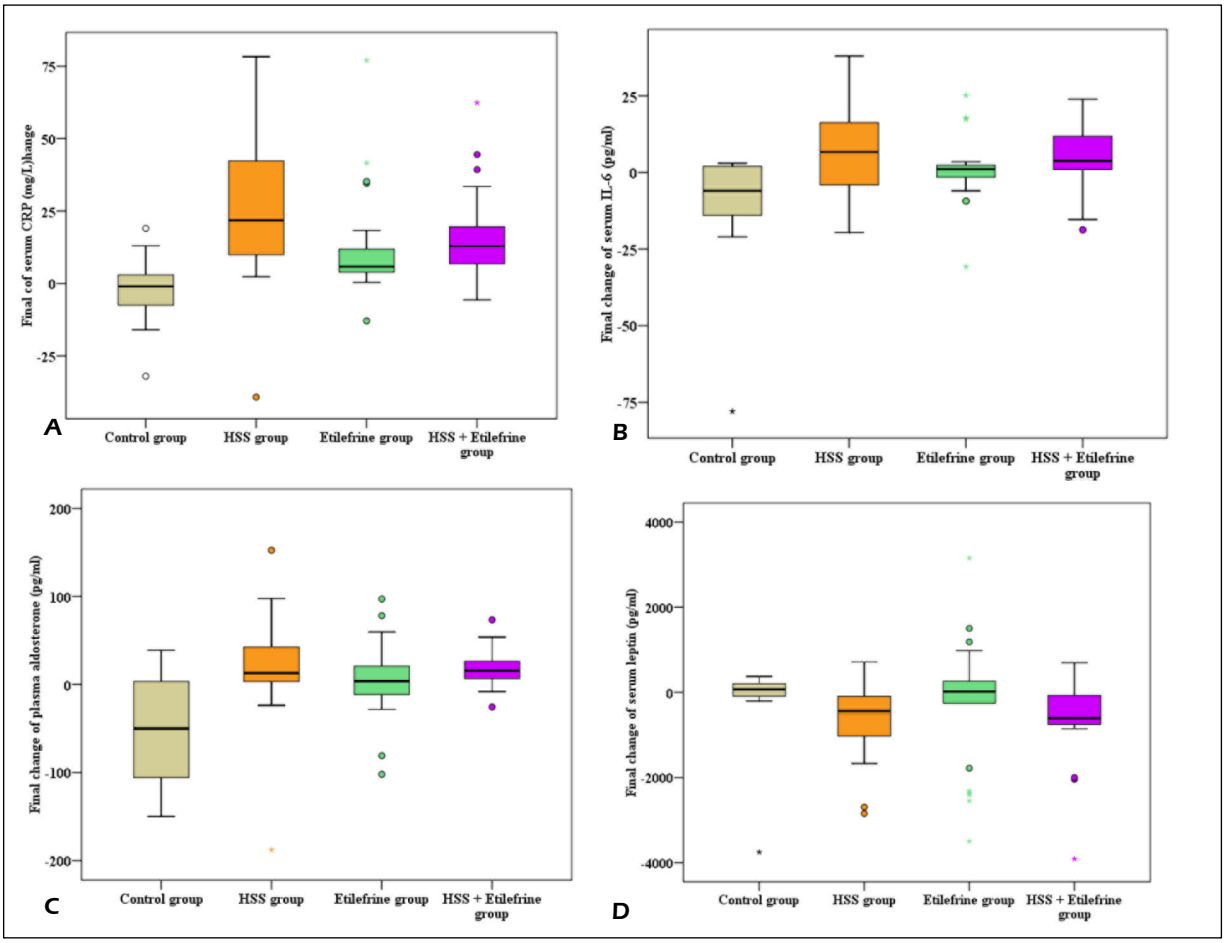

Figure 4. Effect of treatment regimens on the biological markers. **A**, Serum CRP concentrations. **B**, Serum IL-6 concentrations. **C**, Plasma aldosterone concentrations. **D**, Serum leptin concentrations. The figure shows a significant increase in the serum CRP in the SDT group with a significant decrease in the other groups, while serum IL-6 and aldosterone showed a significant decrease in HSS and combination groups without a significant change in SDT or etilefrine groups. Serum leptin showed a significant increase in HSS and combination groups without a significant increase in the SDT or etilefrine groups. Final changes are calculated by the difference between baseline values – after 38 days values.

referred to the reduction of renal function with chronic diuretic use to increase Na reabsorption and decreased natriuresis, which is associated with activation of RAAS, as we noted a significant reduction of plasma aldosterone in HSS, and combination groups compared to a non-significant increase in SDT group. Contrary to previous data, while the reduction in aldosterone levels in the etilefrine group was significant compared with the SDT group, the reduction was non-significant in the intragroup analysis[7]. This may be referred to as the non-significant effect of etilefrine on serum Na correction despite the improvement of natriuresis, Crcl, urine output, and urinary creatinine concentration.

Furthermore, our finding showed a significant reduction of serum CRP in HSS and combination groups, which agrees with another finding[14,16]. The etilefrine group noted a significant reduction in serum CRP levels with a non-significant reduction in serum IL-6. On the other hand, significant CRP level increase and non-significant serum IL-6 increase in SDT patients were noted. This may be attributed to increased diuresis, which could increase the opsonic capacity of ascites, which may help protect these patients from infections[17].

One hundred and forty patients from 12 liver units in France contributed to a randomized study to evaluate the effect of salt-restricted and unrestricted diet (21 mmol Na) on cirrhotic patients with ascites showed that the ascites resolution, improvement of nutritional status, and hospitalization time in the two groups were the same[17]. We observed a significant increase in serum leptin in HSS and combination groups compared with the non-significant change in the SDT or etilefrine groups, referred to as the HSS effect.

Data correlated changes in body weight in cirrhotic patients with ascites with the changes in ascites volume as an objective marker of ascites improvement[14,18]. Our results are consistent with those of the previous studies as we observed a significant reduction in mean body weight within HSS, etilefrine, and combination groups compared to a non-significant increase in the SDT group. Bodyweight reduction is considered to reflect ascites improvement. Cirrhotic patients with arterial hypertension are less sensitive to developing vasodilatory complications such as hepatorenal and hepatopulmonary syndrome. MAP is an independent predictor of survival in cirrhotic patients with ascites and had one-year survival of 40% with MAP < 82 mmHg compared to 70% for those with MAP > 82 mmHg[19], and low MAP in HBV-cirrhotic patients with ascites is an independent risk factor of death[20].

Our results show a significant elevation of MAP within HSS, and combination groups compared to a non-significant reduction in SDT patients; however, an unexpected non-significant elevation of MAP in the etilefrine group was observed contrary to the results of the previous studies[7,21]; this may be attributed to its β1- and β2 adrenergic agonist activities compared to the pure α1- adrenergic agonist activities of midodrine.

Limitations

This study is based on a small number of patients, and more patients are needed to confirm the study findings. Furthermore, the follow-up duration was short, which is not enough to evaluate the long-term success of treatments. Convincing patients to salt infusion was an excellent challenge for us. The patient's reluctance to participate in the study for consideration related to salt intake impacted the number of participants, especially since the study was in one center, perhaps repeating the study in more than one center supports the positive results more clearly. Moreover, the study was conducted during the COVID-19 pandemic, which affected the number of participants.

Conclusions

Our results suggest that HSS infusion, oral etilefrine, or both plus SDT are superior to SDT alone without hepatic, renal, or hematological abnormalities. Our treatment models revealed a significant reduction in ascites grades based on the reduction of serum aldosterone and enhanced diuresis, Crcl, and urinary Na excretion compared with SDT.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Authors' Contributions
H. Radwan was involved in the study concept, and design, patient enrollment, manuscript writing, data interpretation, and analysis; O. Ibrahim was involved in supervision, managed the study analysis, manuscript writing, and critical reviews; S. El-Hagar was involved in supervision, conception, data interpretation, study analysis, drafting the manuscript; G. Badra was involved in supervision, study design, patient enrollment, critical reviews, and study conduct; H. El-Said was involved in supervision, laboratory analysis, and interpretation of study results. All authors revised and approved the final manuscript.

Data Availability
The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Trial Registration
The trial was registered on ClinicalTrials.gov with the identifier NCT04785755.

Ethics Approval
The study was approved by the Institution Review Board (IRB) of NLI, Menoufia University, Egypt, with NLI/IRB protocol number: 00131/2017, and the Research Ethics Committee of the Faculty of Medicine, Tanta University, approval code: 32624/10/18. The study's design and methods were consistent with the Helsinki Declaration and its subsequent amendments in 1964.

Informed Consent
Informed consent was obtained from all individual participants included in the study.

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