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Effect of Candesartan and Ramipril on Liver Fibrosis in Patients with Chronic Hepatitis C Viral Infection: A Randomized Controlled Prospective Study

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ABSTRACT

Objective: This study aimed at evaluating the effects of candesartan and ramipril on liver fibrosis in patients with chronic hepatitis C.

Methods: This randomized controlled prospective study involved 64 patients with chronic hepatitis C and liver fibrosis. Participants were randomized into 3 groups: group I (control group; n = 21), members of which received traditional therapy only; group 2 (ramipril group; n = 21), members of which received traditional therapy plus 1.25 mg/d oral ramipril; and group 3 (candesartan group; n = 22), members of which received traditional therapy plus 8 mg/d oral candesartan. Patients were assessed at baseline and 6 months after intervention through measuring of liver stiffness (Fibro-Scan; Echosens, Paris, France); evaluation of the serum levels of hyaluronic acid and transforming growth factor beta-1; and calculation of indices of liver fibrosis, including fibrosis index based on the 4 factors and aspartate transaminase-to-platelet-ratio index. Data were analyzed using paired *t* test and 1-way ANOVA followed by Tukey's honest significant difference test for multiple pairwise comparisons.

Results: At baseline, the 3 study groups were statistically similar in demographic and laboratory data. After treatment, the 3 study groups showed significant decrease in liver stiffness, serum levels of hyaluronic acid and transforming growth factor beta-1, and indices of liver fibrosis compared with baseline data ($P < 0.001$). Six months after treatment, patients taking ramipril and candesartan showed significant improvement in all measured parameters compared with the control group. Additionally, the candesartan-treated group showed significant decrease in liver stiffness, biomarkers, and indices of liver fibrosis compared with ramipril recipients.

Conclusions: The administration of ramipril and candesartan in patients with chronic hepatitis C with hepatic fibrosis was well tolerated and effective in improving liver fibrosis. angiotensin II receptor 1 (AT1) antagonist candesartan maintained antifibrotic effects more effectively than ramipril and may represent a safe and effective therapeutic strategy for liver fibrosis in patients with chronic liver diseases. ClinicalTrials.gov identifier: NCT03770936. (*Curr Ther Res Clin Exp*. 2022; 83:XXX–XXX) © 2022 Elsevier HS Journals, Inc.

Introduction

Egypt is the most affected nation by hepatitis C virus.[1] According to the Egyptian ministry of health, Egyptian patients living with viral hepatitis have an increased risk of fibrosis, cirrhosis, and liver cancer. Liver fibrosis is characterized by excess production and deposition of extracellular matrix proteins, including collagen. Some authors reported that the renin angiotensin system (RAS) could play an important role in the progression of liver fibrosis.[2,3] components are overexpressed during hepatic fibrosis, particularly angiotensin II that could promote activation of the hepatic stellate cells (HSCs), the major source for collagen production during chronic liver damage.[4] Given the role of angiotensin

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II in promoting liver fibrosis, renin angiotensin system inhibitors (RASIs), angiotensin-converting enzyme inhibitors (ACE-Is), and angiotensin receptor-1 (AT-1) blockers (ARBs) have been shown to exert protective effects against liver fibrosis.[5] These effects are due to suppression of HSCs transformation into hepatic myofibroblasts and reduced expression of transforming growth factor beta-1 (TGF-β1).[6]

Although, some preclinical studies and experimental models of liver fibrosis demonstrated the antifibrotic effects of both ACE-Is and ARBs,[7] the reported results are conflicting, which may be attributed to the heterogeneity of disease models, drugs, and doses used.[8] Also, the data obtained from clinical studies about the antifibrotic effect of ACE-Is and ARBs were obscure and controversial.[9–11] Moreover, this avid interest in RASIs is related to their relative safety in human beings and their widespread use in cardiovascular and renal diseases.

In this context, this study aimed at evaluating the antifibrotic effects of an ACE-I (ramipril) and an ARB (candesartan) on liver fibrosis in patients with chronic hepatitis C through determination of liver stiffness (Fibro-Scan; Echosens, Paris, France); evaluation of serum levels of biomarkers of liver fibrosis, including hyaluronic acid (HA) and TGF-β1; and calculating the indices of liver fibrosis such as fibrosis index based on the 4 factors (FIB-4) and aspartate transaminase-to-platelet ratio index (APRI).

Patients and Methods

Study design and patient selection

This randomized, controlled, prospective study was conducted on patients with chronic hepatitis secondary to hepatitis C virus infection. Sixty-four patients with liver fibrosis secondary to viral hepatitis C were recruited from Hepatitis C Unit, National Liver Institute of Institutional University Hospital Shebin El-Kom, Menoufia governorate, Egypt, between October 2018 and September 2019 and were included in the final analysis. The participants were randomized in a 1:1:1 ratio using computer-generated code according to the consolidation standards of reporting trials guidelines into 1 of the following groups: group I (control group; n = 21), which received traditional therapy composed of sofosbuvir 400 mg/d in combination with daclatasvir 60 mg/d, and ribavirin 1000 or 1200 mg/d based on body weight; group 2 (ramipril group; n = 21), which received traditional therapy plus a daily oral dose of 1.25 mg ramipril for 6 months; and group 3 (candesartan group; n = 22), which received traditional therapy plus a daily oral dose of 8 mg candesartan for 6 months. Diagnosis of fibrosis was based on clinical, laboratory, and Fibro-Scan data. All patients were classified as F3 or F4 (Fibro-Scan $\geq$ 9.5 kPa). The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments. The institutional Research Ethics Committee approved the current study. The institutional Research Ethics Committee of both Tanta University and Menoufia University approved the current study. The study was registered as clinical trial (ClinicalTrials.gov identifier: NCT03770936). Written informed consents were taken from all participants.

Inclusion criteria were aged between 18 and 75 years, patients with liver fibrosis without history of decompensation, patients without esophageal varices, patients without ascites, and those who were not receiving diuretics therapy. The exclusion criteria were patients with hepatitis B infection, hepatocellular carcinoma, anemia, thrombocytopenia, thalassemia, acute hepatitis, cholestasis, esophageal varices, hypotension, cardiomyopathy, renal dysfunction (creatinine $>$ 1.5 mg/dL and creatinine clearance $<$ 40 mL/min), portal vein thrombosis, and diabetes. Patients taking vasoactive drugs were excluded. Patients using ACE-Is or ARBs for other conditions were also excluded from the study. For all participants in the 3 study groups, complete blood count, liver function tests, liver stiffness (via Fibro-Scan), and biomarkers of liver fibrosis were assessed at enrollment (baseline) and at the end of the study.

Sample collection

Approximately 10 mL venous blood was withdrawn from each patient by sterile venipuncture, without frothing and after minimal venous stasis using disposable syringes. The blood sample was divided into 3 parts for the biochemical analysis of the measured parameters.

Measurement of hematological parameters

About 2 mL blood was transferred into vacutainer tube containing potassium EDTA for the assessment of hemoglobin level and for the determination of complete blood count (Sysmex XN-1000; Sysmex America Inc, Lincolnshire, Illinois).

Measurement of liver function parameters

About 3 mL of the collected venous blood was introduced to vacutainer serum tubes. Centrifugation at 3000 rpm was done immediately to avoid sample contamination by erythrocyte arginase. Sera were used for the determination of liver function. Serum alanine aminotransferase (ALT) (reference value = 7–55 U/L) and serum aspartate aminotransferase (AST) (reference value = 8–48 U/L) were determined spectrophotometrically using kinetic method.[12] Total and direct serum bilirubin levels (reference value = 0.3–1.2 mg/dL and 0.3 mg/dL, respectively) were assayed spectrophotometrically using colorimetric (Diazo) method.[13] Serum albumin concentration (reference range = 3.5 to 5.0 g/dL) was determined spectrophotometrically using modified bromocresol green colorimetric method.[14] Kits used for biochemical analysis were supplied by (Siemens Healthcare Diagnostics Products Gmbh, Marburg, Germany).

Measurement of liver stiffness

Liver stiffness measurement was performed using transient elastography (via Fibro-Scan) depending upon the method formerly described.[15] The liver stiffness measurement was performed by a single experienced and well-trained operator. At least 10 valid measurements were obtained for each patient. Results were included in the final analysis only if the following 3 criteria were met: at least 10 valid measurements, success rate $>$ 60% (success rate represented by the ratio of the number of valid measurements and total shots that has to be at least $>$ 60%) and the interquartile range-to-liver-stiffness ratio was $\leq$ 0.30 (interquartile range shows the variability between the 10 valid determinations, which is shown to not exceed 30% of the mean; ie, final results). The median values of the validated measurements for each patient were representative of the liver stiffness. The fibrosis result is measured in kilopascals (normal value $<$ 7 kPa for healthy people without liver diseases).

Analysis for biomarkers of liver fibrosis

About 3 mL collected venous blood was drawn into EDTA tubes for the measurement of serum HA and TGF-β1. These tubes were kept refrigerated before blood sample collection. Sera were separated within 30 minutes after blood withdrawal and kept frozen at

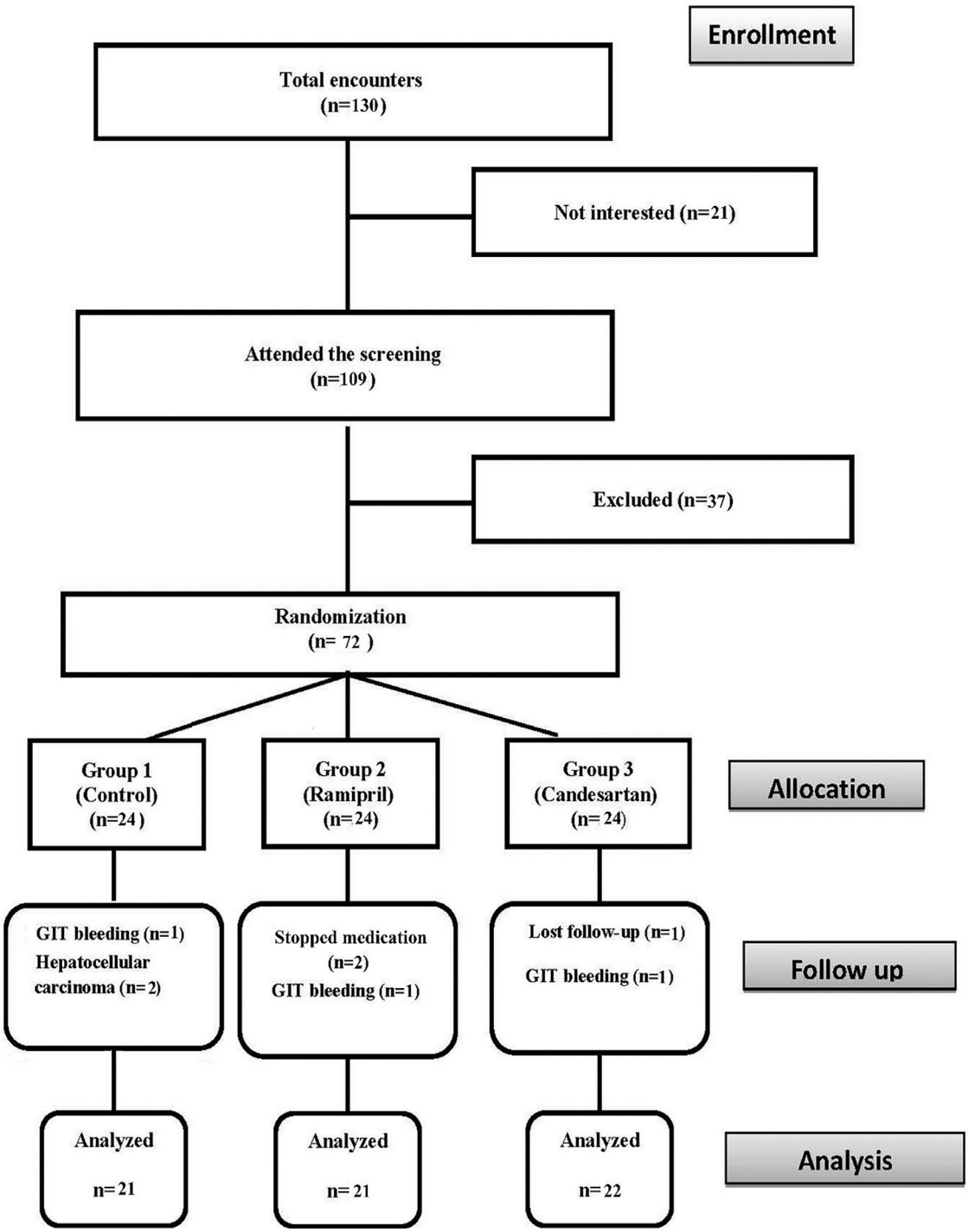

Figure 1. Flowchart illustrating the participants' screening, enrollment, and randomization. GIT = gastrointestinal.

−80°C until the quantitative determination of HA and TGF-β1 levels, which were assayed by a double antibody sandwich ELISA using commercially available ELISA kits and in accordance with the manufacture instructions (SunRed; SunRed Biological Technology Co Ltd, Shanghai, China).

Calculation of indices of liver fibrosis

The fibrosis indices, including fibrosis index based on FIB-4, APRI were calculated according to the following formulas: The formula used for FIB-4 is: age (years) × AST (IU/L)/[platelet count (10^9/L) × $\sqrt{}$ ALT (IU/L)].[16] The formula used for APRI is: AST (IU/L)/(upper limit of normal) × 100/platelet count (10^9/L).[17]

Subjective data analysis

Patients were followed-up through monthly direct meeting and were monitored weekly by telephone calls to assess their correct use of the study medication, evaluate their compliance, and report any medications-related adverse reactions. Patients were withdrawn from the study if any adverse reactions related to the candesartan or ramipril were found in the monthly evaluations such

Table 1
Demographic data of the study participants.

Parameter	Group 1: Control (n = 21)	Group 2: Ramipril (n = 21)	Group 3: Candesartan (n = 22)	*P* value*
Age†, y	56.52 (6.64)	57.09 (6.93)	55.96 (9.03)	0.887
Weight†, kg	77.86 (3.26)	78.62 (4.41)	78.05 (3.72)	0.798
Height†, m	1.70 (0.05)	1.69 (0.05)	1.70 (0.06)	0.677
Body mass index†	26.97 (1.68)	27.73 (2.50)	27.03 (1.79)	0.402
Male sex‡	12 (57.14)	13 (61.90)	14 (63.64)	0.904

* $P < 0.05$ is considered significant.
† Values are presented as mean (SD).
‡ Values are presented as n (%).

Table 2
Baseline selected laboratory data of all patients in the 3 study groups.*

Parameter	Group 1: Control (n = 21)	Group 2: Ramipril (n = 21)	Group 3: Candesartan (n = 22)	*P* value†
WBCs, 10^{3}/μL	6.53 (0.90)	6.69 (1.20)	6.86 (1.38)	0.657
Lymphocytes, 10^{3}/μL	2.13 (0.69)	2.50 (0.55)	2.40 0.45)	0.103
Platelets, 10^{3}/μL	154.43 (45.57)	158.33 (54.82)	160.23 (40.13)	0.919
Hb, g/dL	13.67 (1.48)	13.56 (1.07)	13.75 (1.36)	0.900
PT, sec	13.76 (0.67)	13.49 (1.04)	13.76 (1.48)	0.666
PC, %	81.05 (7.49)	85.29 (14.76)	83.14 (11.07)	0.494
INR	1.22 (0.18)	1.21 (0.21)	1.11 (0.15)	0.117
SBP, mm Hg	124.29 (6.18)	124.52 (10.94)	123.41 (10.51)	0.920
DBP, mm Hg	83.09 (11.12)	84.05 (9.44)	82.46 (6.63)	0.851
ALT, IU/L	29.67 (7.43)	31.43 (7.74)	30.14 (6.03)	0.707
AST, IU/L	23.71 (7.83)	30.43 (8.58)	32.14 (6.39)	0.603
BIL-T, mg/dL	0.80 (0.20)	0.80 (0.19)	0.79 (0.15)	0.981
BIL-D, mg/dL	0.28 (0.14)	0.26 (0.08)	0.24 (0.07)	0.533
Albumin, g/dL	4.23 (0.33)	4.11 (0.50)	4.29 (0.35)	0.344
Liver stiffness,(kPa	19.04 (4.06)	18.48 (4.25)	18.21 (3.01)	0.773
HA, ng/mL	192.75 (31.47)	188.12 (6.54)	194.01 (38.56)	0.874
TGF-β1, ng/mL	31.03 (8.61)	31.30 (6.54)	30.54 (7.70)	0.947
FIB4	2.47 (1.12)	2.18 (0.89)	2.14 (0.60)	0.433
APRI	0.75 (0.36)	0.65 (0.22)	0.65 (0.14)	0.339

ALT = alanine aminotransferase; APRI = aspartate transaminase-to-platelet ratio index; AST = aspartate transaminase; BIL-D = direct bilirubin; BIL-T = total bilirubin; DBP = diastolic blood pressure; FIB-4 = fibrosis index based on the 4 factors; HA = hyaluronic acid; Hb = hemoglobin; INR = international normalized ratio; kPa = kilopascal; PC = prothrombin concentration; PT = prothrombin time; SBP = systolic blood pressure; TGF-β1 = transforming growth factor-b1; WBCs = white blood cells.
* Values are presented as mean (SD).
† $P < 0.05$ is considered significant.

as hypotension (blood pressures $\leq$ 90/60 mm Hg), hyperkalemia (serum potassium level $>$ 5.5 mmol/L), angioedema, dry cough, or other reported side effects, and then the standard treatments that suit each case were supplied. Patients' adherence to the study medication was assessed through counting the tablets and through the medications refill rate. A patient was considered nonadherent when he/she underused, overused, or discontinued the study medications. The underuse of medications is the administration of less than the prescribed amount of the medications, which can result in underestimation of the measured efficacy. The overuse of medication is the administration of more than the prescribed amount of the medication, which may result in drug-related adverse effects, which in turn can force patients to either stop the medications or have poor adherence.

Statistical Analysis

All data were analyzed using IBM-SPSS statistical package version 24.0 (IBM-SPSS Inc, Armonk, New York). Kolmogorov-Smirnov test was used to assess the normality of data. The χ^2 test was used for statistical analysis of nominal data. Paired *t* test was used to assess any significant difference between each group at baseline and 6 months after treatment. One-way ANOVA test was used to assess any significant difference between the 3 study groups at baseline and 6 months after treatment followed by Tukey's honestly significant difference test for multiple pairwise comparisons. Correlation between the measured variables was performed using Pearson correlation analysis. Data were presented as mean (SD), number, and percent. Significance level was set at ($P < 0.05$).

Results

Figure 1 illustrates the participant flowchart. Out of 130 patients with liver fibrosis screened, 21 patients were not interested in participating and 37 patients were excluded according to the study exclusion criteria and the remaining 72 patients who fulfilled the inclusion criteria were enrolled and randomized into the 3 study groups. During the follow-up period, 8 patients dropped out and were omitted from the final analysis (1 patient was lost to follow-up, 2 patients stopped medications, 2 patients developed hepatocellular carcinoma, and 3 patients showed severe gastrointestinal bleeding). In this context, only 64 patients completed the study and their data were analyzed.

As shown in Table 1, all groups were statistically similar regarding their demographic data, including age, sex, weight, height, body mass index, sex, and smoking habit. At baseline, there was a nonstatistical difference in the selected laboratory features of all participants in the 3 study groups ($P > 0.05$) as shown in Table 2.

Compared with baseline data, 6 months after intervention, patients in the 3 study groups showed statistically significant decrease in ALT, AST, total bilirubin, HA, and TGF-β1 serum levels. The 3 study groups showed also significant decline in indices of liver fibrosis (ie, FIB-4 and APRI) and liver stiffness, which was associated with significant elevation in albumin level (paired *t* test, $P < 0.05$). These aforementioned data with all *P* values are demon-

Table 3
The measured parameters of the 3 study groups at baseline and after treatment.*

Parameter	Group 1: Control (n = 21)		(P) paired *t* test	Group 2: Ramipril (n = 21)		(P) paired *t* test	Group 3: Candesartan (n = 22)		(P) paired *t* test*
	Before	After		Before	After		Before	After	
WBCs, $10^3/\mu L$	6.53 (0.9)	6.91 (0.8)	< 0.001†	6.69 (1.2)	6.99 (1.22)	< 0.001†	6.86 (1.38)	7.79 (1.18)	< 0.001†
Lymphocyte, $10^3/\mu L$	2.13 (0.7)	2.17 (0.6)	0.446	2.50 (0.6)	3.20 (0.72)	< 0.001†	2.40 (0.45)	2.80 (0.48)	0.001†
Platelets, $10^3/\mu L$	154.43 (45.6)	166.91 (42.8)	< 0.001†	158.33 (54.8)	180.09 (51)	< 0.001†	160.2 (40.13)	230.2 (44.2)	< 0.001†
Hb, g/dL	13.67 (1.5)	12.70 (1.5)	< 0.001†	13.56 (1.1)	12.66 (0.95)	< 0.001†	13.75 (1.4)	12.87 (1.27)	< 0.001†
PT, sec	13.76 (0.7)	11.93 (0.9)	< 0.001†	13.49 (1.04)	12.25 (0.71)	< 0.001†	13.76 (1.5)	12.06 (1.32)	< 0.001†
PC, %	81.05 (7.5)	87.48 (8.9)	< 0.001†	85.29 (14.8)	90.81 (13.2)	0.001†	83.14 (11.07)	90.05 (10.2)	< 0.001†
INR	1.22 (0.2)	1.09 (0.1)	0.003†	1.21 (0.2)	1.07 (0.07)	0.006†	1.11 (0.15)	1.06 (0.07)	0.046†
SBP, mm Hg	124.29 (6.2)	122.86 (9.2)	0.428	124.52 (10.9)	118.81 (11.2)	0.003†	123.4 (10.5)	117.3 (9.09)	< 0.001†
DBP, mm Hg	83.09 (11.1)	82.86 (8.5)	0.927	84.05 (9.4	76.43 (8.39)	< 0.001†	82.46 (6.6)	75.46 (6.16)	< 0.001†
ALT, IU/L	29.67 (7.4)	24.48 (6.5)	< 0.001†	31.43 (7.7)	20.09 (5.79)	< 0.001†	30.14 (6.03)	16.05 (4.11)	< 0.001†
AST, (IU/L)	32.71 (7.8)	26.76 (5.4)	< 0.001†	30.43 (8.6)	22.19 (5.34)	< 0.001†	23.14 (6.39)	18.27 (5.06)	< 0.001†
BIL-T, mg/dL	0.80 (0.2)	0.76 (0.2)	0.016†	0.80 (0.19)	0.72 (0.17)	0.001†	0.79 (0.15)	0.59 (0.17)	< 0.001†
BIL-D, mg/dL	0.28 (0.1)	0.24 (0.2)	0.181	0.26 (0.08)	0.23 (0.10)	0.158	0.24 (0.07)	0.21 (0.07)	0.085
Albumin, g/dL	4.23 (0.3)	4.46 (0.3)	0.001†	4.11 (0.50)	4.56 (0.24)	< 0.001†	4.29 (0.35)	4.65 (0.26)	< 0.001†
Liver stiffness, kPa	19.04 (4.1	16.45 (3.7	< 0.001†	18.48 (4.25)	13.02 (3.20)	< 0.001†	18.21 (3.01)	9.08 (2.05)	< 0.001†
HA, ng/mL	190.75 (31.5	170.68 (35.9)	< 0.001†	188.12 (45.6)	131.68 (44.3)	< 0.001†	194 (38.56)	102.99 (29.6)	< 0.001†
TGF-β1, ng/mL	31.03 (8.6	24.74 (9.4)	< 0.001†	31.30 (6.5)	16.73 (5.32)	< 0.001†	30.54 (7.70)	11.28 (5.01)	< 0.001†
FIB4	2.47 (1.1	1.99 (0.8)	< 0.001†	2.18 (0.89)	1.69 (0.53)	0.001†	2.14 (0.60)	1.18 (0.47)	< 0.001†
APRI	0.75 (0.4	0.55 (0.2)	< 0.001†	0.65 (0.22)	0.40 (0.09)	< 0.001†	0.65 (0.14)	0.25 (0.07)	< 0.001†

ALT = alanine aminotransferase; APRI = The aspartate transaminase-to-platelet ratio index; AST = aspartate transaminase; BIL-D = direct bilirubin; BIL-T = total bilirubin; DBP = diastolic blood pressure; FIB-4 = the fibrosis index based on the 4 factors; HA = hyaluronic acid; Hb = hemoglobin; INR = international normalized ratio; kPa = kilopascal; PC = prothrombin concentration; PT = prothrombin time; SBP = systolic blood pressure; TGF-β1 = transforming growth factor-b1; WBCs = white blood cells.

* Values are presented as mean (SD).

† Significant at $P < 0.05$.

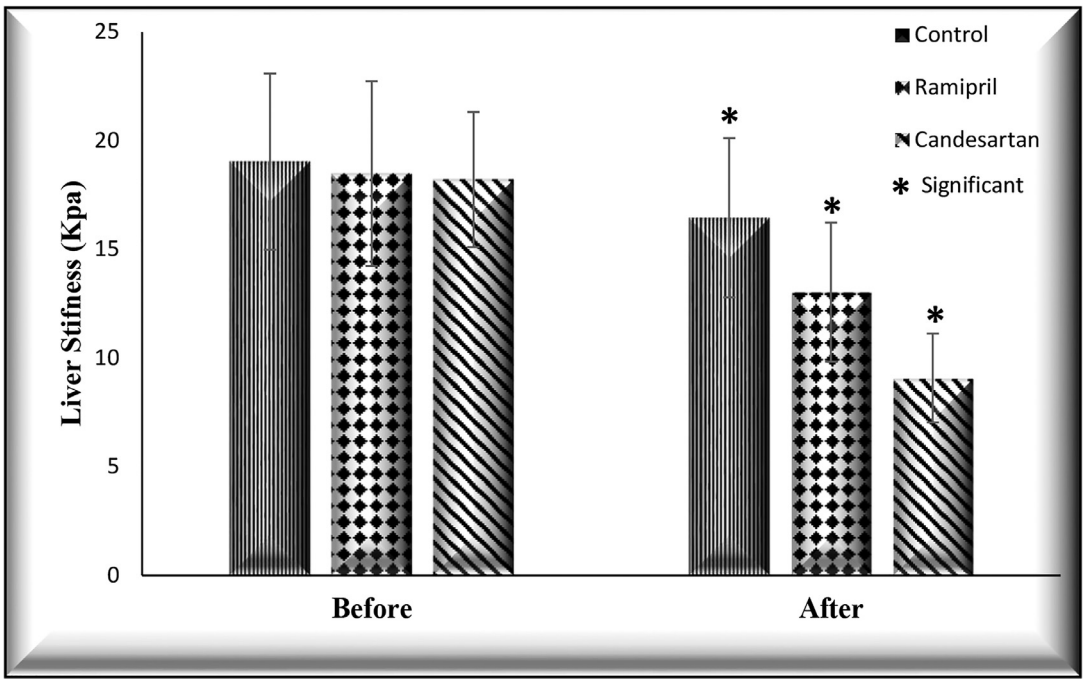

Figure 2. Changes in liver stiffness in the 3 studied groups before treatment and 6 months after treatment. Liver stiffness in both ramipril and candesartan groups decreased significantly ($P < 0.001$) 6 months after treatment in comparison with its baseline. Values are presented as mean (SD). *Significant difference.

strated in **Table 3**. **Figures 2**, **3**, and **4** illustrate the changes in liver stiffness, HA, and TGF-β1 in the 3 study groups before and 6 months after treatment.

Table 4 demonstrates the comparison between the 3 study groups 6 months after treatment. Compared with the control group (group 1), the ramipril-treated group (group 2) showed significantly lower ALT, AST, liver stiffness, HA, TGF-β1, and APRI index ($P = 0.034$, $P = 0.018$ $P = 0.002$, $P = 0.003$, $P = 0.001$, and $P = 0.004$, respectively). On the other hand, the candesartan-treated group (group 3) showed significantly lower ALT, AST, HA, TGF-β1 levels, liver stiffness, and indices of liver fibrosis compared with the control group ($P < 0.001$). The comparison between ramipril- and candesartan-treated groups revealed that 6 months after treatment, the candesartan-treated group (group 3) showed significantly lower ALT, AST, HA, TGF-β1 levels, FIB-4 index, and APRI index compared with the ramipril-treated group ($P = 0.041$, $P = 0.047$, $P = 0.036$, $P = 0.030$, $P = 0.019$, and $P = 0.005$, respectively). In addition, the candesartan-treated group showed significant decline in liver stiffness compared with the ramipril-treated group ($P < 0.001$). **Figure 5** illustrates the comparison between the effects produced by the 3 therapeutic options on liver stiffness.

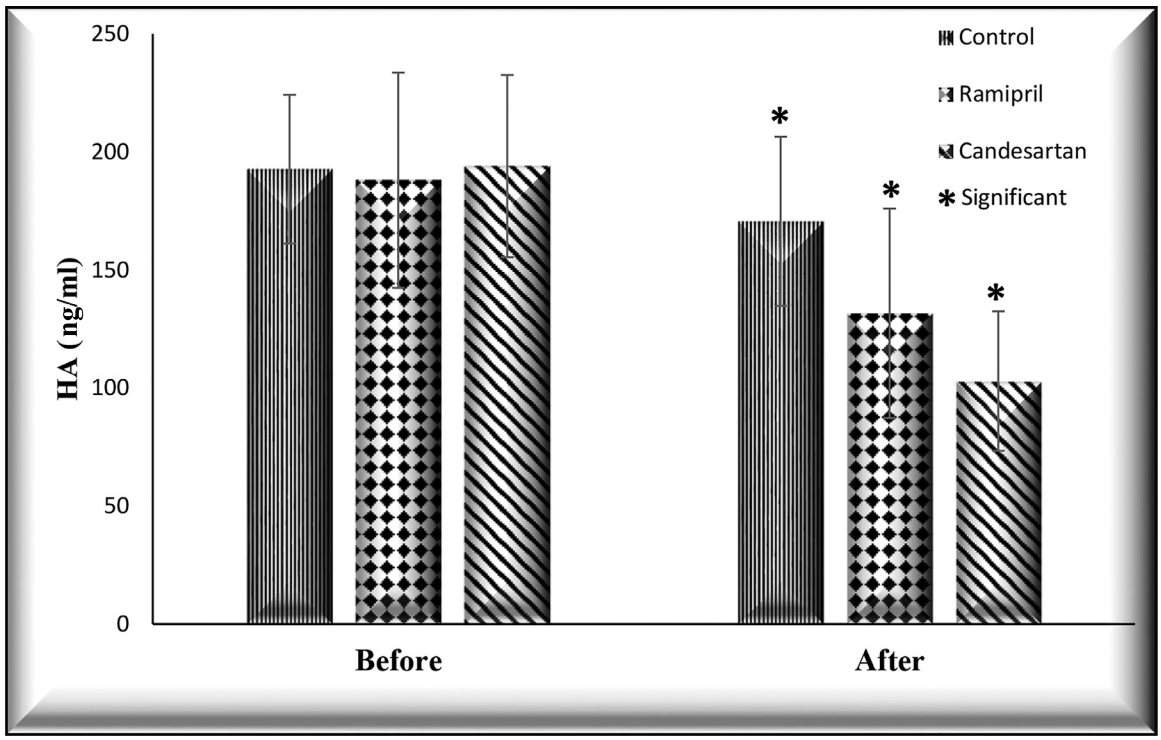

Figure 3. Changes in hyaluronic acid (HA) serum level in the 3 studied groups before treatment and 6 months after treatment. HA level in both ramipril and candesartan groups decrease significantly ($P < 0.001$) 6 months after treatment in comparison with its baseline level. Values are presented s mean (SD). *Significant difference.

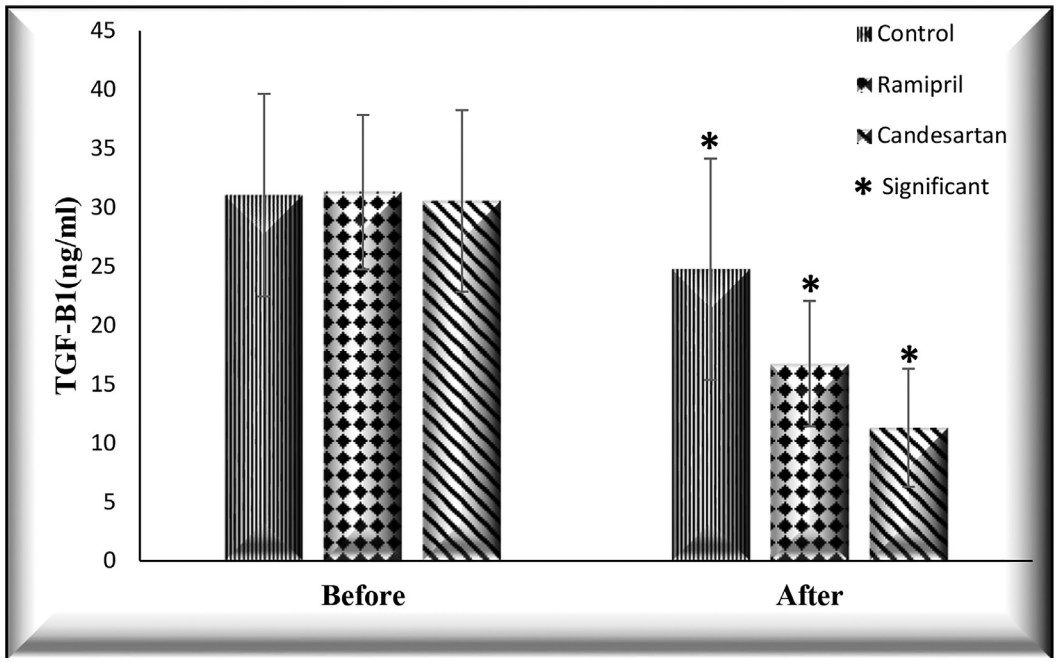

Figure 4. Changes in transforming growth factor-beta 1 (TGF-β1) serum level in the 3 studied groups before treatment and 6 months after treatment. TGF-β1 level in ramipril and candesartan groups decreased significantly ($P < 0.001$) 6 months after treatment in comparison with its baseline level. Values are presented as mean ± SD. *Significant difference.

Figure 6 illustrates the Pearson correlation analysis between the measured variables, which revealed presence of significant positive correlation between HA, TGF-β1, FIB-4, and APRI index with liver stiffness ($r = 0.348$ [$P = 0.022$], $r = 0.480$ [$P = 0.001$], $r = 0.360$ [$P = 0.018$], and $r = 0.642$ [$P < 0.001$], respectively) after treatment. Furthermore, we observed presence of significant positive correlation between TGF-β1 and HA ($r = 0.488$ [$P = 0.001$]).

Safety and tolerability of the study medications

Regarding drug-related complications, no serious adverse reactions were observed in any patients of the study groups. Only 1 patient in the ramipril group experienced cough versus no one in the other study groups ($P = 0.36$). During the study period, no one in the 3 study groups developed hyperkalemia, hypotension,

Table 4
Comparison among the 3 study groups 6 months after treatment.*

Parameter	Group 1: Control (n = 21)	Group 2: Ramipril (n = 21)	Group 3: Candesartan (n = 22)	ANOVA	Tukey HSD test		
					Group 1: Control (n = 21)	Group 2: Ramipril (n = 21)	Group 3: Candesartan (n = 22)
WBCs, 10^3/μL	6.91 (0.84)	6.99 (1.22)	7.79 (1.18)	0.020†	0.969	0.030‡	0.054§
Lymphocytes, 10^3/μL	2.17 (0.64)	3.20 (0.72)	2.80 (0.48)	< 0.001†	< 0.001‖	0.005‡	0.091
Platelets, 10^3/μL	166.91 (42.76)	180.09 (51.00)	230.18 (44.18)	< 0.001†	0.625	<0.001‡	0.002§
Hb, g/dL	12.70 (1.51)	12.66 (0.95)	12.87 (1.27)	0.842			
PT, sec	11.93 (0.86)	12.25 (0.71)	12.06 (1.32)	0.588			
PC, %	87.48 (8.86)	90.81 (13.16)	90.05 (10.20)	0.584			
INR	1.09 (0.08)	1.07 (0.07)	1.06 (0.07)	0.370			
SBP, mm Hg	122.86 (9.16)	118.81 (11.17)	117.27 (9.09)	0.169			
DBP, mm Hg	82.86 (8.45)	76.43 (8.39)	75.46 (6.16)	0.005†	0.024‖	0.007‡	0.910
ALT, IU/L	24.48 (6.51)	20.09 (5.79)	16.05 (4.11)	< 0.001†	0.034‖	< 0.001‡	0.041§
AST, IU/L	26.76 (5.44)	22.19 (5.34)	18.27 (5.06)	< 0.001†	0.018‖	< 0.001‡	0.047§
BIL-T, mg/dL	0.76 (0.21)	0.72 (0.17)	0.59 (0.17)	0.006†	0.731	0.007‡	0.050§
BIL-D, mg/dL	0.24 (0.15)	0.23 (0.10)	0.21 (0.07)	0.621			
Albumin, g/dL	4.46 (0.34)	4.56 (0.24)	4.65 (0.26)	0.100			
Liver stiffness, kPa	16.45 (3.66)	13.02 (3.20)	9.08 (2.05)	< 0.001†	0.002‖	< 0.001‡	< 0.001§
HA, ng/mL	170.68 (35.86)	131.68 (44.32)	102.99 (29.56)	< 0.001†	0.003‖	< 0.001‡	0.036§
TGF-β1, ng/mL	24.74 (9.40)	16.73 (5.32)	11.28 (5.01)	< 0.001†	0.001‖	< 0.001‡	0.030§
FIB-4	1.99 (0.75)	1.69 (0.53)	1.18 (0.47)	< 0.001†	0.212	< 0.001‡	0.019§
APRI	0.55 (0.23)	0.40 (0.09)	0.25 (0.07)	< 0.001†	0.004‖	< 0.001‡	0.005§

ALT = alanine aminotransferase; APRI = the aspartate transaminase-to-platelet ratio index; AST = aspartate transaminase; BIL-D = direct bilirubin; BIL-T = total bilirubin; DBP: diastolic blood pressure; FIB-4 = the fibrosis index based on the 4 factors; HA = hyaluronic acid; Hb = hemoglobin; HSD = Honestly Significant Difference; INR = international normalized ratio; kPa = kilopascal; PC = prothrombin concentration; PT = prothrombin time; SBP = systolic blood pressure; TGF-β1: transforming growth factor-beta 1.

* Values are presented as mean (SD) unless otherwise noted.

† Significant at $P < 0.05$.

‡ Significant difference group 3 versus group 1.

§ Significant difference group 3 versus group 2.

‖ Significant difference group 2 versus group 1.

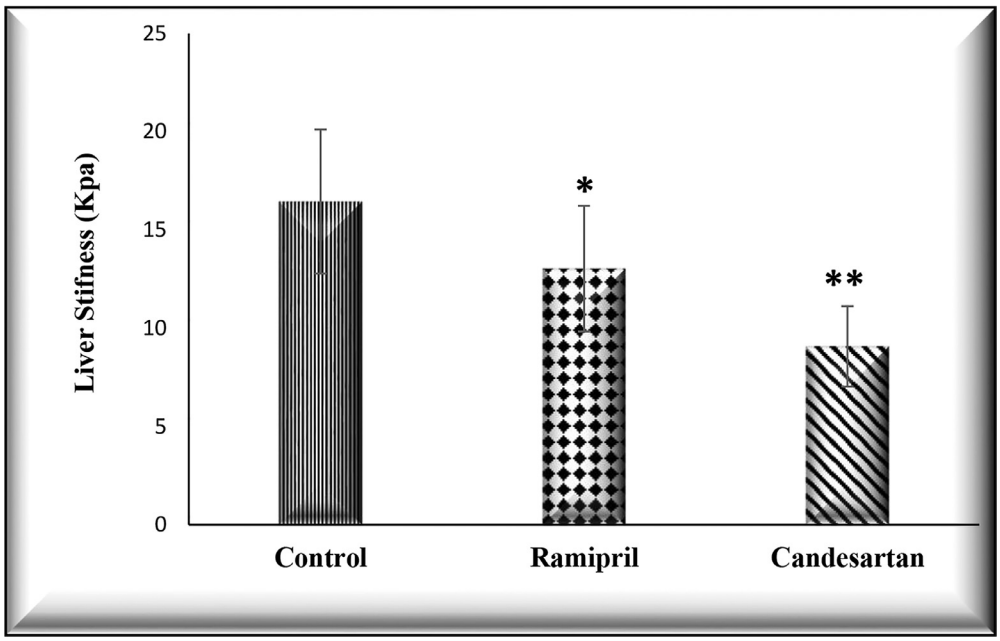

Figure 5. Liver stiffness of the 3 studied groups after treatment. Values are presented a mean (SD). *Significant difference from control group. **Significant difference from control and ramipril groups

angioedema, nor anemia (decrease in hemoglobin level by ≥ 2 g/L). Four patients in the control group versus 5 patients in the ramipril group and 3 patients in the candesartan group developed controllable gastrointestinal symptoms ($P = 0.71$). Finally, 1 patient in the candesartan group developed manageable flu-like symptoms ($P = 0.36$).

Discussion

The RAS was reported to be involved in liver fibrosis.[18] Additionally, RASIs were reported to have a potential role in attenuating hepatic fibrosis.[2,5,6] However, clinical data about the effects of RASIs on liver fibrosis are inconsistent.[9–11] Hence, we aimed at evaluating the effects of ACE-I (ramipril) and ARB (candesartan) on liver fibrosis in patients with chronic hepatitis C.

During the current study, the etiology of fibrosis for all enrolled patients was viral hepatitis C, which seems in agreement with a previous report.[19] Our study duration was 6 months, which seems acceptable and matches some previous studies.[20] there was no standardization for ACE-I or ARB doses, low doses of ramipril (1.25 mg/d) and candesartan (8 mg/d) were scheduled in the cur-

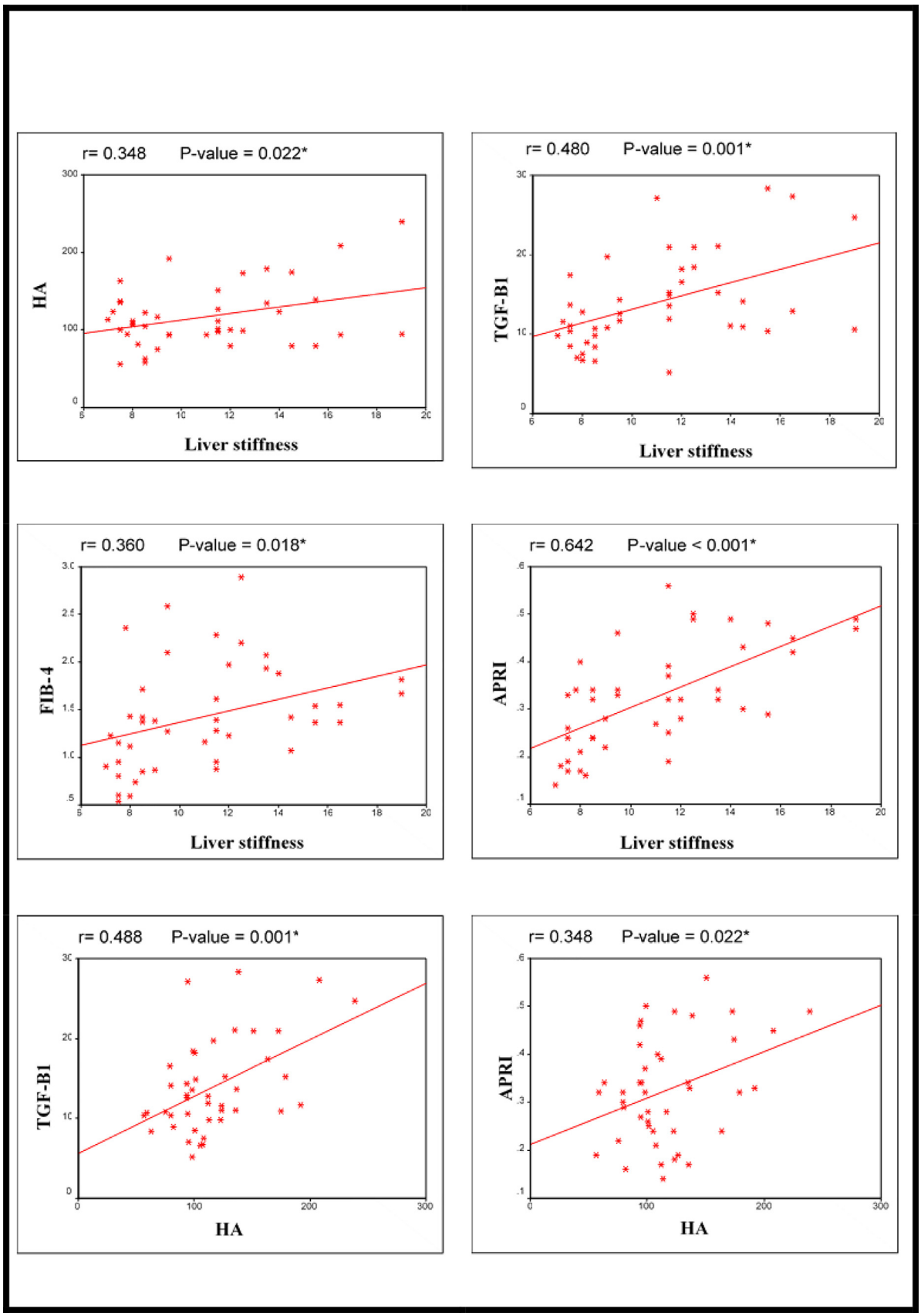

Figure 6. The Pearson correlation analysis between the measured variables. APRI = aspartate transaminase-to-platelet ratio index; FIB-4 = fibrosis index based on the 4 factors; HA = hyaluronic acid; TGF-β1 = transforming growth factor-beta 1.

rent study to avoid problems associated with high doses, including hypotension that could impair liver perfusion with subsequent worsening of liver fibrosis.

Although liver biopsy remains the gold standard for detecting hepatic fibrosis, biopsy is highly invasive and it is associated with considerable risk for sampling error.[21] Therefore, we based our study on noninvasive and safe methods for the assessment of liver fibrosis, which included measurement of liver stiffness (via Fibro-Scan), evaluation of serum levels of biomarkers of liver fibrosis (ie, HA and TGF-β1) and calculation of FIB-4 and APRI indices. Fibro-Scan has a diagnostic accuracy in detecting liver fibrosis, with specificity and sensitivity being reported to approach 90%.[22] HA and TGF-β1 have been demonstrated as the most useful diagnostic and prognostic noninvasive biomarkers for hepatic fibrosis.[23,24] Furthermore, FIB-4 and APRI indices were reported to have a diagnostic accuracy for detecting liver fibrosis.[25]

The 3 study groups were statistically similar at baseline; therefore, any changes that occurred after treatments were attributed to the outcomes of the study medications. At the end of this study and compared with the control group, both ramipril and candesartan produced significant improvement in liver enzymes (ie, ALT and AST), serum albumin level, and liver fibrosis, which was translated by significant decrease in liver stiffness, serum levels of biomarkers of liver fibrosis (ie, HA and TGF-β1), and indices of liver fibrosis.

In fact, RAS components are overexpressed in hepatic fibrosis, especially angiotensin II. The fibrogenic effect of angiotensin II can be mediated through activating AT1. Angiotensin II can induce both HSCs proliferation and Kupffer cells activation, both of which are implicated in fibrogenesis.[4,26–28] Angiotensin II was also reported to upregulate TGF-β1 mRNA expression in Kupffer cells.[29,30] Furthermore, there is a positive feedback in the liver, whereas TGF-β1 activates HSCs, and HSCs can produce much more TGF-β1.[31,32] Therefore, angiotensin II could promote hepatic fibrosis through activation of HSC via TGF-β1, among the most profibrotic cytokines that accumulate extracellular matrix and its noncollagenous glycoproteins, including HA.[3,33] Moreover, angiotensin II acts as a proinflammatory mediator through increasing the tumor necrosis factor-alpha mRNA expression by Kupffer cells.[34] In this context, the beneficial effects of both ramipril and candesartan on liver function and liver fibrosis may be attributed to their ability to block the RAS, especially angiotensin II.[3,34]

The improvement in liver fibrosis obtained after 6 months of administration of both ramipril and candesartan seems in accordance with previously reported findings demonstrated that AT1-receptor blocker losartan may inhibit the progression of liver fibrosis in patients with chronic hepatitis C.[5,20] Our results seem also in consonance with some previous reports postulated therapeutic efficacy of RASIs on liver fibrosis.[9]

The reduction in liver stiffness produced by both ramipril and candesartan was associated with significant decline in the circulating levels of biomarkers of fibrogenesis (ie, HA and TGF-β1). This significant decrease in the serum level of TGF-β1 confirms the role of angiotensin II in stimulating the production of growth factors, including TGF-β1 and connective tissue growth factor, and hence the migration and accumulation of activated HSCs at the site of hepatic injury. The decrease in the serum level of HA could be explained on the basis that RASIs could suppress HSC and decrease TGF-β1 with subsequent decrease of extracellular matrix and its components, including HA.

The beneficial effects of both ramipril and candesartan on liver fibrosis and TGF-β1 are compatible with former studies postulated that RAS blocking by either ACE or AT1 inhibition resulted in attenuation of liver fibrosis through suppression of HSCs and TGF-β1.[6,9,35] Our previous result seems in agreement with former studies demonstrating that HA, TGF-β1, and other profibrotic cytokines were decreased by ARBs.[35,36] Furthermore, our result comes in parallel with a previous preclinical study demonstrated that ARB and/or rifaximin treatments reduced hepatic TGF-β1 levels and consequently hepatic fibrogenesis in rat model of nonalcoholic steatohepatitis.[37] However, our results are in conflict with some previously reported studies demonstrating that ACE-Is/ARBs did not retard the progression of hepatic fibrosis therapy in patients with chronic hepatitis C and HIV and hepatitis C coinfection.[38,39]

Nonalcoholic steatohepatitis can result in hepatic fibrosis, cirrhosis, and hepatocellular carcinoma.[40] The pathological changes in nonalcoholic fatty liver disease, including nonalcoholic steatohepatitis, may at least partly result from the activation of the inflammatory arm of RAS.[41] However, our results are in contradiction with some former studies conducted on patients with nonalcoholic steatohepatitis revealing that ARB therapy failed to improve liver fibrosis and suggesting there is insufficient evidence to support the efficacy of ARBs in managing hepatic fibrosis in patients with nonalcoholic steatohepatitis.[10,11] These conflicting results may be attributed to diversity of the study designs and their duration, heterogeneity of the disease models, heterogeneity of the studied populations, and variability of drugs used and their dosages.

During the current study, the effect produced by candesartan on liver stiffness (liver fibrosis), biomarkers of liver fibrosis (ie, TGF-β1 and HA) and liver panel was significantly higher than the effect produced by ramipril. The significantly higher antifibrotic effect of candesartan over ramipril could be explained on the basis that the AT1-receptor antagonist candesartan can completely block the deleterious effect of angiotensin II at AT1-receptor level. On the other hand, ramipril as an ACE-I has no action on AT1-receptor level and provokes the accumulation of bradykinin, which may decrease the beneficial effect of ramipril on liver fibrosis. Bradykinin was reported to play a role in fibrosis through stimulating the proliferation of mesangial cells and through activation of TGF-β1.[42] Additionally, bradykinin was reported to provoke immune liver injury in mice through bradykinin type 2 receptors mediated pathway and through blocking of bradykinin type 1 receptor, which attenuates immune liver injury.[43,44] Our former result could be supported by the previously reported finding demonstrated that ARBs showed more effectiveness in suppressing hepatic fibrosis compared with ACE-I in animals.[45] Furthermore, Zhu et al,[9] in their meta-analysis of randomized controlled trials demonstrated that ARBs were widely used and seemed effective in attenuating the liver fibrosis with good safety profile and nonsignificant withdrawals.

We observed presence of significant positive correlation between serum TGF-β1 with both HA and liver stiffness. This reported correlation is compatible with some previous findings demonstrated that serum level of HA was positively associated with both serum TFG-β1 and TGF-β1 expression in the selected consecutive liver compartments.[46,47] In addition, the expression of TGF-β1 was reported to be closely associated to liver fibrosis and evaluation of serum TGF-β1 levels may contribute to the diagnosis of liver fibrosis.[48] We reported also the existence of positive significant correlation between serum HA and liver stiffness, a result seems in accordance with the findings of other authors who reported that serum HA levels are related to stage of fibrosis and degree of necroinflammation.[23]

Regarding drug safety and tolerability, the study medications were well tolerated with no evidence of serious adverse reactions. During the study period, none of hyperkalemia, hypotension, angioedema, or anemia was reported among the participants of the 3 study groups. Some patients in the 3 study groups developed controllable gastrointestinal side effects during the initial period of treatment. The majority of these gastrointestinal side effects dis-

appeared with the time. Only 1 patient in the ramipril group developed cough during the follow-up period and discontinued the medication and therefore his data were omitted from the final analysis. The safety and tolerability of the current study medications seems in accordance with a former pilot study demonstrated the safety and efficacy of 6 months' administration of losartan in patients with chronic hepatitis C and liver fibrosis.[20]

A point of strength of the current study concerns its priority as a first clinical trial directed to compare the efficacy of candesartan versus ramipril on liver fibrosis. However, the small sample size represents a limitation for the current study.

Study limitations

Despite our promising results, our study has some limitations, including relatively small sample size and being open label. Therefore, further large-scale studies are still needed.

Conclusions

The current study established that the administration of ramipril and candesartan in patients with chronic hepatitis C and hepatic fibrosis for 6 months was well tolerated and effective in improving liver fibrosis. Both ramipril and candesartan produced significant improvement in liver function associated with significant decline in liver stiffness and serum levels of biomarkers of liver fibrosis. The AT1-receptor antagonist candesartan maintained antifibrotic more effectively than ramipril and may represent a safe and effective therapeutic strategy for liver fibrosis in patients with chronic liver diseases.

Conflicts of Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

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Tarek M. Mostafa, Gamal A. El-azab, and Abeer A. Elsayed reviewed the literature and constructed the study design. Eligibility assessment, enrolment of participants, as well as randomization and collection of clinical data were performed by Gamal A. Badra and Alyaa S. Abdelwahed. Tarek M. Mostafa and Abeer A. Elsayed performed laboratory and statistical analysis. All authors read, revised, and approved the final manuscript.

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