Myocardial ischemia, cerebral ischemia and myocardial infarction are among the most important complications of hypertension and atherosclerotic disease in developing countries. Angiotensin converting enzyme (ACE) inhibitors are among the drugs used to treat hypertension and heart failure. Captopril is an ACE-inhibitor which also has antioxidant properties. This study was conducted to assess the antioxidant effects of Iranian Captopril on malondialdehyde (MDA), conjugated dienes (CD) and serum antioxidant capacity before and after treatment.

METHODS: This interventional prospective single-blind study was conducted on 34 mildly hypertensive individuals and 34 patients with stage I and II heart failure. MDA, CD and serum antioxidant capacity were measured in all samples. The patients were then given 50 mg Captopril tablets 2-3 times daily. The measurements were repeated 1.5 months later.

RESULTS: Comparison of mean MDA, CD and serum antioxidant capacity in hypertensive patients and patients with heart failure before and after drug administration revealed no significant difference in any of the parameters studied.

DISCUSSION: Existing evidence is suggestive of the strong antioxidative properties of Captopril in vitro, although these effects have not been borne out by some studies. In the present study, comparison of MDA, CD and serum antioxidants before and after the period of treatment with Iranian Captopril did not reveal any statistically significant difference.

Keywords • Antioxidant • ACE inhibitor • High blood pressure • Heart failure • Clinical trial

Captopril is considered as a routine and standard treatment for congestive HF.\(^{12,13}\) It is usually administered orally to control hypertension. Captopril mediates its effects through inhibiting the activity of angiotensin converting enzyme (ACE). It also effectively inhibits the conversion of dehydralase. Captopril has a biological half-life of about two hours and exerts its antihypertensive effects over a period of six hours. Captopril reduces blood pressure in patients with primary hypertension, as well as those with renal vascular problems. It also improves the prognosis of congestive HF patients by increasing cardiac output.\(^{14, 15}\) Captopril also seems to have antioxidant properties.\(^{11, 16, 17}\)

This study was conducted to evaluate the antioxidant effects of Captopril, by measuring malondialdehyde (MDA), conjugated dienes (CD) and serum antioxidant capacity before and after a course of treatment with Captopril.

**Materials and methods**

This interventional prospective single-blind study was carried out as a clinical trial involving 34 patients with mild hypertension and 34 patients with stage I and II HF based on diagnostic criteria cited in Braunwald's Textbook of Heart Disease\(^{18}\) and the Framingham study.\(^{19, 20}\) MDA, CD and serum antioxidant capacity were measured in all samples prior to treatment. The patients were administered 50mg Captopril tablets (BID or TID) produced by Lorestan Pharmaceutical Company. All of the tests were repeated 1.5 months later and mean values were compared between the two groups using paired t-test. Data collection was as follows:

a) **Measurement of MDA using thiobarbituric acid:** 0.5 cc citrated plasma was added to 1 cc chloroacetic acid and centrifuged at 4200 rpm for 10 minutes. 0.5 millimeter of the resultant supernatant was removed, mixed with 0.67% thiobarbituric acid and placed in a Bain-Marie double boiler at 100ºC for ten minutes. MDA was measured at the wavelength of 532 nanometers.\(^{21}\)

b) **Measurement of antioxidant capacity:** Blood samples were eluted and 20% erythrocyte suspension was prepared. 50 µl of diluted plasma was added to 200 µl erythrocyte suspension and placed in Bain-Marie double boiler at 37ºC for ten minutes. In the next stage, 200 µl of 40 millimolar AAPH solution was added to the suspension and was placed in Bain-Marie double boiler with shaker at 37ºC for two hours. The suspension was then removed from the boiler and 1 cc sodium phosphate buffer was added; it was centrifuged and the photoabsorption of the supernatant fluid was measured at the wavelength of 540 nanometers.\(^{22}\)

c) **Measurement of conjugated dienes:** 100 µl citrated plasma was added to 1 cc distilled water and thoroughly shaken. Then chloroform and methanol (2 cc chloroform + 1 cc methanol) were added. The solution was shaken for two minutes and centrifuged for five minutes at 3000 rpm. The supernatant was discarded and the remaining watery fluid was put in a separate tube and left to vaporize in a Bain-Marie double boiler at 40ºC. The remnant was dissolved in 1 cc hexane and the photoabsorption of the fluid was measured at the wavelength of 234 nanometers.\(^{23}\)

d) **Blood pressure measurement:** Blood pressure measurement was conducted by a cardiologist at the clinic of Isfahan Cardiovascular Research Center according to WHO standards.\(^{21}\)

**Statistical methods.** Data analysis was performed using SPSS/win software program. Comparison of MDA, CD, and serum antioxidant capacity in each group was performed using paired t-test before and after the study.

**Results**

A total of 34 HF patients (9 women and 25 men) with a mean age greater than 60 years and 34 hypertensive patients (17 men and 17 women) with a mean age of 70 years were studied.

### TABLE 1. Comparison of mean levels of malondialdehyde (MDA), antioxidant capacity and conjugated dienes (CD) before and after administration of Captopril to patients with hypertension and HF

<table>
<thead>
<tr>
<th>Patients</th>
<th>MDA (µmol/lit)</th>
<th>Antioxidant capacity</th>
<th>CDs (µmol/lit)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Mean±SD</td>
<td>After Mean±SD</td>
<td>P*</td>
</tr>
<tr>
<td>Heart failure</td>
<td>1.3287±0.27</td>
<td>1.2775±0.23</td>
<td>0.463</td>
</tr>
<tr>
<td>(N=34)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.32±0.31</td>
<td>1.35±0.26</td>
<td>0.788</td>
</tr>
<tr>
<td>(N=34)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P value in paired t-test
Mean diastolic and systolic blood pressure was 81.11±13.64 mmHg and 131.11±24.72 mmHg respectively in the HF group, and 102.38±22.36 mmHg and 162.22±18.32 mmHg respectively in the hypertensive group.

Table 1 represents a comparative assessment of mean MDA, CD, and serum antioxidant capacity in HF and hypertensive patients before and after Captopril administration; the results showed no significant difference.

Table 2 represents a comparative assessment of mean MDA, CD, and serum antioxidant capacity in HF and hypertensive patients before and after Captopril administration according to sex; the results showed no significant difference.

**Discussion**

Existing evidence is suggestive of the potent anti-oxidative properties of Captopril in vitro, while other ACE inhibitors do not display such effects. Given that such effects have not been borne out by some studies, the present study was conducted to compare MDA, CD and serum antioxidant capacity in HF and hypertensive patients before and after a course of treatment with Captopril; no significant statistical results were observed in this study.

An empirical study conducted on mice with experimentally-induced hypertension evaluated the effect of common antihypertensive drugs, namely Captopril, hydralazine and prazosin on antioxidant tissue enzymes and lipids.

All three drugs led to marked increase in Cu/Zn superoxidase and reduction of glutathione peroxidase. Captopril increases the activity of superoxidase (Mn type) as well as catalase, hence affecting damaged myocardial tissue. Studies on the activity of liver and muscle enzymes have shown that the resultant changes are often specific.

Another study has demonstrated that Captopril and enalapril increase the antioxidant capacity in a number of tissues, ultimately leading to morphological and functional changes related to the aging process. Total plasma antioxidant capacity increases slightly in subjects under treatment with Captopril.

Captopril increases the antioxidant capacity of lipid tissues; it also acts as a perfusion regulator in damaged cardiac tissue. Tensile and resting myocardial forces, as well as contractile forces of the myocardium are affected by Captopril. Having come into contact with ischemia-damaged tissue, Captopril augments superoxidase activity, also benefiting damaged myocardial tissue via anti-free radical mechanisms. Moreover, ACE inhibitors reduce the production of free radicals; their use following electroshock defibrillation or cardiac dysfunction has been shown to reduce free radicals in heart vessels, hence countering the destructive effects of electroshock.

A Japanese study evaluated the effect of Captopril on systolic blood pressure, renal function, and the activity of glutathione peroxidase, superoxidase dismutase, dismutase superoxide, catalase, and oxygen radicals. After twenty weeks, beneficial therapeutic effects were observed on the renal activity of hypertensive patients, as well as on the brain function of hypertensive patients prone to stroke. Results of the study of the effects of Captopril and ascorbic acid on free radicals and peroxidation have shown that ascorbic acid inhibits peroxidation, however, Captopril does not.

Another study conducted on 14 male and female patients with hypertension assessed levels of lipid peroxides, as well as catalase activity in plasma and erythrocytes before and after daily administration of 25-50 mg Captopril for six months.
Lipid peroxide levels and catalase activity were shown to have decreased significantly at the end of the study.26 Captopril apparently protects erythrocytes against hemolysis due to AAPH and hypochlorite, however, enalapril does not seem to have such effects and even in some instances, results in increased hemolysis. The latter study has concluded that only ACE inhibitors with the SH moiety have antioxidant properties.19 Some studies involving hypertensive subjects have demonstrated an increase in lipid peroxidation, lipoproteins, MDA peroxidase and CD compared to controls.28 Clinical studies have shown the positive effects of ACE inhibitors on patients with HF, hence these agents are recommended in the treatment of hypertension and heart failure. ACE inhibitors (enalapril and Captopril) increase the defenses of tissues against antioxidants.27 At the conclusion of the present study, however, no statistically significant difference in MDA, serum antioxidant capacity and CD was observed; this may have been due to ineffectiveness of Iranian-made Captopril on the said parameters.

Many Iranian physicians do not believe strongly in the antihypertensive effects of Captopril. To avoid ethical concerns, we increased drug dosage in 90% of the patients, but the drug did not display any antioxidant effects even at increased doses. The results of this study warrant more expansive studies on drugs produced in Iran, so that the extent of their effectiveness can be assessed via clinical trials.

References