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# The effects of wet cupping on serum high-sensitivity C-reactive protein and heat shock protein 27 antibody titers in patients with metabolic syndrome

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**KEYWORDS**
Metabolic syndrome;
Wet cupping;
Heat shock protein 27;

**Summary**
*Introduction:* It has previously been reported that increased level of serum heat shock proteins (Hsps) antibody in patients with metabolic syndrome. It is possible that the expression of Hsp and inflammatory markers can be affected by cupping and traditional Chinese medicine. There is a little data investigating the effects of cupping on markers of inflammation and Hsp proteins,

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High sensitive C-reactive protein

hence, the objective of this study was evaluation of the effects of wet cupping on serum high-sensitivity C-reactive protein (hs-CRP) and Hsp27 antibody titers in patients with metabolic syndrome.

*Subjects and methods:* Serum Hs-CRP and Hsp27 antibody titers were assessed in samples from 126 patients with metabolic syndrome (18—65 years of age) at baseline, and after 6 and 12 weeks after treatment. One hundred and twenty-six patients were randomly divided into the experimental group treated with wet cupping combined with dietary advice, and the control group treated with dietary advice alone using a random number table. Eight patients in case group and five subjects in control groups were excluded from the study. Data were analyzed using SPSS 15.0 software and a repeated measure ANCOVA.

*Results:* Serum hs-CRP titers did not change significantly between groups ($p > 0.05$) and times ($p = 0.27$). The same result was found for Hsp27 titers ($p > 0.05$).

*Conclusion:* Wet-cupping on the interscapular region has no effect on serum hs-CRP and Hsp27 patients with metabolic syndrome.



## Introduction

Metabolic syndrome is diagnosed by presence of central obesity, dyslipidemia, hypertension and hyperglycemia.[1] The prevalence of metabolic syndrome has been reported to be 25% in United States of America,[2] 17.8—34% in Europe[3,4] and 12.8—41% Asian Pacific and the Middle East.[3,5]

The heat shock proteins also known as stress proteins are found in all cell types and in all organisms.[6] An increased level of serum Hsp family especially Hsp27 is found in subjects with metabolic syndrome and cardiovascular disease.[7—10]

One the other hand, some studies show that cupping and traditional Chinese medicine can affect on Hsp expression and inflammatory markers.[11—13] With regard to, there is a little data to investigate the effectiveness of wet cupping on markers of cardiovascular disease such as hs-CRP as well as Hsp27. Therefore, the objective of this study was evaluation of the effects of wet cupping on serum hs-CRP and Hsp27 antibody titers in patients with metabolic syndrome.

## Methods

### Subjects

This research was designed as a randomized controlled trial study that was approved by the Mashhad University of Medical Sciences Ethics Committee. The patients ($n = 136$, aged 18—65 years) with metabolic syndrome were included in this trial. Patients who had hemophilia, infectious disease, stroke, heart attack, type 1 diabetes, secondary dyslipidemia, renal dysfunction, epilepsy, and pregnancy were excluded from the study. Patients who were following especial drug or dietary regimen were also excluded. We use intention to treat method to deal with the missing data. One hundred and twenty-six patients therefore were included in the study. The patients were randomly assigned into control ($n = 63$) or an experimental group ($n = 63$). The patients in the control group were prescribed a dietary advice that provided a 500 kcal per day less than total energy expenditure for 12 weeks. The patients in experimental groups were advised the same dietary regimen and two times wet cupping at the weeks 1 and 6 after treatment (Fig. 1). For purposes of matching, participants were placed under an isocaloric regimen for 2 weeks. After that, serum hs-CRP and Hsp27 were measured at baseline, 6 and 12 weeks after treatment.

### HS-CRP antibody measurement

HS-CRP was measured by a cobas autoanalyzer at 6 and 12 weeks after treatment.

### Anti-Hsp27 antibody measurement

Serum Hsp27 antibody titers (Stressgen, Canada) were assessed using an in-house enzyme-linked immunosorbent serologic assay (ELISA). A 50 ng recombinant human Hsp27 was dissolved in 50 μl carbonate buffer pH = 9.6 and was placed in per well plate (Nunc Maxisorp, Nunc) at 4 °C in a humidified chamber for overnight. The wells were washed 3 times in buffer phosphate saline (PBS), treated with 0.05% Tween-20. To block non-specific binding, well plates were incubated in 2% goat serum in PBS for 30 min in 37 °C and 30 min at room temperature. Wells were washed 3 times with PBS. After 30 min incubation in serum Hsp27(diluted 1:50) in room temperature, wells were washed three times. Then, well plates were coated with 100 μl peroxide conjugated-goat anti-human IgG (Sigma—Aldrich, USA) for 30 min at room temperature. After being washed twice in PBS, each well was incubated at 100 μl of TMB substrate (100 μl of 6 mg/ml TMB in DMSO and 10 ml of 50 mM acetate buffer pH 4.5 containing 3 μl $H_2O_2$) for 15 min in a dark room. After adding 50 μl 3 M HCl for each well, optical density at 450 nm was read using a Labsystems iEMS Reader MF Microtiter plate reader. The ratio of optical density was compared to a reference wavelength of 620 or 570 nm and results were given in optical density units.

### Wet cupping

A single physician was performed wet cupping for all patients. The vacuum glass was placed on skin overlying

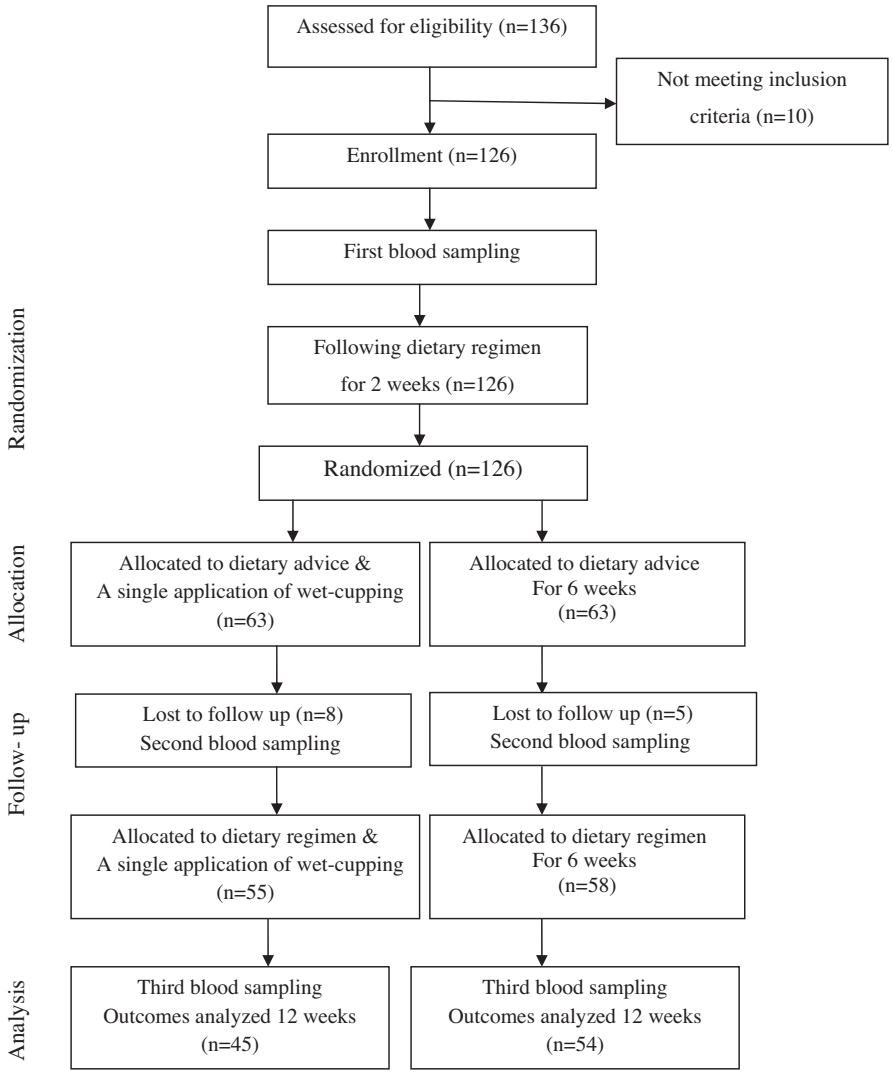


**Fig. 1** CONSORT trial flow-chart.

trapezius muscle, at the level of scapular spine. A vacuum was produced by electrical suction that resulted from anchoring the vacuum glass on to the skin. Then, the skin scarified by a sterile surgical blades and blood was drawn by the vacuum into the glass. The glasses were removed and location of cupping was bandaged.

### Statistics analysis

Data were analyzed using SPSS 15.0 software and a repeated measure ANOVA. After logarithmic transformation on hscrp and hsp27 repeated measure ANCOVA test was used. A $p < 0.05$ was considered significant.

## Results

At start, demographic characteristics and hs-CRP, hsp27 baselines were compared between experimental and control groups. The results showed that age ($p = 0.365$), education ($p = 0.616$), marriage status ($p = 0.75$), job ($p = 0.43$), hs-CRP baseline ($p = 0.23$) and hsp27 baseline ($p = 0.99$) were not significant difference between two groups. Also there was no interaction between time with hs-CRP and time with hsp27.

### Serum hs-CRP and Hsp27 antibody titers

Table 1 shows serum hs-CRP and Anti-Hsp27 titers of experimental and control groups. There was no significant difference between values of hs-CRP in control and experimental groups ($p = 0.58$) as well as within times ($p = 0.27$). A reduction in hs-CRP values of control groups was observed in compared to baseline, however this reduction was not significant ($p > 0.05$). A 17.07% reduction between week 0 and 6 and a 2.43% increase between weeks 6 and 12 was found in hs-CRP values of experimental groups ($p > 0.05$).

### Safety

There were no adverse effects in any subjects.

**Table 1** Comparison of serum hsCRP and Anti-Hsp27 levels between the experimental and control groups.

| Parameter | Experimental group ($N = 45$) | | | Control group ($N = 54$) | | | ANCOVA[a] ($p$-value) |
|---|---|---|---|---|---|---|---|
| | Baseline | Week 6 | Week 12 | Baseline | Week 6 | Week 12 | |
| hs-CRP (mg/L) | 4.1 ± 3.5 | 3.4 ± 3.0 | 4.2 ± 3.4 | 4.8 ± 3.60 | 4.6 ± 3.7 | 4.2 ± 2.7 | 0.270 |
| Changes (%) | | 17.07 | 2.43 | | 4.16 | 12.5 | 0.270 |
| Anti-Hsp27 (AU) | 104.7 ± 10.5 | 103.1 ± 10.0 | 98.9 ± 8.4 | 104.9 ± 11.9 | 103.7 ± 12.2 | 101 ± 12.0 | <0.001 |
| Changes (%) | | 1.52 | 5.53 | | 1.14 | 3.71 | <0.001 |

*Note*: Values expressed as mean ± SD. hs-CRP, high sensitive-C-reactive protein; AU, absorbance unit; Hsp, heat shock protein.
[a] Adjusted for the baseline.

## Discussion

This project aimed to investigate the effects of cupping therapy on serum Hsp27 and hs-CRP concentration in patient with metabolic syndrome. Our results showed that level of both serum hs-CRP and Hsp27 concentration did not different significantly in the cupping group compared to the diet group. Maybe the sample size is too small to evaluate the effect properly.

With regard to our knowledge, there is a little data to investigate the effects of cupping on markers of inflammation and Hsp proteins. Cupping may consider as a therapy with low price and no complication in patients with metabolic syndrome. Besides, cupping may use for treatment mild cases instead of drug therapy to prevent side effects of drugs. On the other hands, because of ethical problems, there was wet cupping combined with a diet treatment and we could not have a cupping group alone until demonstrated effects of cupping separately. In addition, result from financial support; there was not a phlebotomy group. It is better that examine different methods of wet cupping with higher sample size in the future studies.

### Effects of cupping on serum Hsp concentration evaluated in animal model

Liu et al. in an experimental study evaluated effects of bloodletting therapy on levels of interleukin-1 and Hsp70 in arthritic model animal using radioimmunoassay.[12] Thirty-two arthritic model rats were selected randomly and were divided into four groups: control, model, bloodletting, and acupuncture group. The results showed no significant difference between Hsp70 level of control, acupuncture and bloodletting group but Hsp70 increased in model group compared to the control group. Also, a high level of interlukin-1 was observed when compared to the control group. The level of interlukin-1 in the bloodletting and acupuncture groups was higher than the control group, but lower than interlukin-1 level of the model group.[12] Hence, bloodletting can improve inflammation damage via regulation of Hsp70 and interlukin-1. In consist, the results of our study demonstrated that there was no significant difference between serum levels of Hsp27 between experimental and control groups.

Ahmed and colleagues in Al-Azhar University was investigated effects of bloodletting cupping therapy on interlukin-2, natural killer cells and hs-CRP in patients with rheumatoid arthritis. Thirty subjects randomly divided into three groups (control group: normal subjects without treatment, experimental group 1: patients with rheumatoid arthritis treated with conventional therapy, and experimental group 2: patients with rheumatoid arthritis treated with conventional therapy combined bloodletting therapy). A significant reduction in interlukin-2, natural killer cells and hs-CRP were observed in experimental group 2 compared to the baseline. There were no significant changes these markers in experimental group 1.[11]

In another study, the biochemical and hematological factors compared between cupping blood and normal venous blood. Fifty-six healthy men (aged 20—40 years) included to the study. Their findings showed a significant difference

among low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), uric acid, triglyceride, iron, white blood cell (WBC), red blood cell (RBC), hemoglobin and alkaline phosphates but not in hs-CRP level. Although in our study, there was not a blood donation group but the serum levels of LDL-C, HDL-C, triglyceride, hs-CRP were difference in cupping group compared to the control one.[14]

This study had several limitations. Because of ethical problems, there was wet cupping combined with a diet treatment and we could not have a cupping group alone until demonstrated effects of cupping separately. In addition, result from financial support; there was not a phlebotomy group.

## Conclusion

Wet-cupping on the interscapular region has no effect on serum hs-CRP and Hsp27 patients with metabolic syndrome.

## Conflict of interest

None declared.

## Acknowledgement

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