

The effect of traditional wet cupping therapy on methylarginine load

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Hi-jama therapy or wet cupping therapy is a complementary therapy that has been in order to centuries in the complementary and preventive treatment of various cardiovascular, metabolic, and inflammatory disorders. However, its mechanism of action is yet not completely elucidated and further studies are needed. This study aims to add to the elucidation of the hi-jama therapy mechanism by comparing methylarginine grades in cupping blood and intravenous blood. This study included 100 healthy women aged 20-60 years, without any chronic disease, who underwent hi-jama therapy at Konya Necmettin Erbakan University Medical Faculty Traditional and Complementary Medicine Center. Methylarginine measurement was performed as a biochemical test with the tandem mass spectrometry. It was determined that total methylarginine concentrations increased in cup blood compared to venous blood in individuals who underwent wet cupping therapy. Our findings support the hypotheses that wet cupping therapy may act through detoxification of harmful metabolites from the blood.

Keywords: ADMA, Arginine, Cupping therapy, Methylarginine

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Cupping therapy is an ancient healing technique based on the principle of applying a sub-atmospheric pressure through heat or suction by applying cups to determined skin points¹. Cupping therapy has been used in China, Korea and East Asia as a traditional and alternative therapy method for the treatment of different medical conditions. However, in recent years, cupping therapy has become increasingly popular in Europe and the United states². Although cupping therapy is a very old technique dating back to 1550 BC, today it is used as an alternative and complementary method in the treatment of medical conditions such as musculoskeletal diseases characterized with aches and pains, cardiovascular disorders, skin diseases, inflammatory disorders, neuropsychiatry problems and metabolic disorders³. Cupping therapy is classified as wet and dry cupping. In both techniques, suction is applied to acupuncture areas, painful areas or reflex zones with the help of cups made of plastic, bamboo or glass. Dry cupping performed with a slight vacuum is created on the skin without drawing blood while wet cupping therapy include markup little quantities of blood and extracellular fluid from the person, usually after composing minor incisions or superficial skin

abrasions⁴. The operation of steps of cupping therapy is yet not fully understood. However, it is suggested that it is effective by promoting peripheral blood circulation and strengthening immunity with the pressurized suction process created in the cupping area. Studies have shown that cupping therapy can reduce inflammatory substances while increasing immunomodulatory compounds^{1,2}. It is also considered to stimulate thymus activity, assist the neuroendocrine system and strengthen the body's antioxidant defence line⁵. In addition, wet cupping therapy is reported to remove heavy metals, toxins, oxidant compounds or substances such as uric acid, cholesterol, urea, and triglycerides, which are found in high concentrations in the body⁶. Methylarginine differentiation like asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), and L-N-monomethyl arginine (L-NMMA) are released by proteolytic catalysis by breaking the methylation of the arginine residue in proteins. It has been reported that methylarginines are involved in the management of various diseases such as cardiovascular illnesses, metabolic disorders, chronic kidney diseases, and cerebrovascular diseases by reducing nitric oxide (NO)

levels⁷. Although cupping therapy has been shown to have beneficial effects on cardiovascular, metabolic and inflammatory diseases in which methylarginine is involved, the mechanism of action has not yet been elucidated⁸. Therefore, our aim in this research is to contribute towards understanding the mechanism of action investigating the results of cupping therapy on methylarginine pathway.

Material and Methods

Study design

Participants

This study included 100 healthy women aged 20-60 years, without any chronic disease, who underwent hi-jama therapy at Konya Necmettin Erbakan University Medical Faculty Traditional and Complementary Medicine Center Turkey. Individuals with hemoglobin levels below 9.5 mg/dL, cardiovascular, autoimmune, renal, cerebrovascular disorders, liver damage, malignant, psychiatric disorders, cigarette smokers, and those who were taking antioxidants, vitamins mineral supplements and alcohol were excluded from the study. In addition, male subjects were not included in the research due to the restricted number of volunteers. Immediately after the participants' 12 h fasting blood samples were taken into serum separator gel tubes and whole blood samples drawn into ethylenediaminetetraacetic acid (EDTA) tubes, wet cupping therapy was applied to the participants and blood samples were transferred to separate plasma sizer tubes. Serum samples were centrifuged at 2000 g for 10 min, and then divided plasma specimens were aliquoted and stored at -80°C up to analysis time. The research was accepted by Selcuk University local ethics committee (Number: 2022/172, Date: 29/03/2022).

Wet cupping therapy

It was performed with the help of the aseptic suction mugs on acupuncture tip regions. The selection of cupping application points was made as the weakest points of the lymphatic system on our back, which includes acupuncture meridians (sun cupping).

Laboratory parameters

The plasma concentrations of total cholesterol, triglycerides, high-density lipoprotein-cholesterol (HDL-C), very low-density lipoprotein (VLDL-C),

urea, creatinine, glucose, alanine aminotransferase (ALT) and aspartate amino transferase (AST) were measured with a Roche Cobas 8000 modular analyzer (Roche® Diagnostics, Switzerland) according to manufacturer's instructions. Low-density lipoprotein (LDL-C) was planned using Friedewald's formula⁹. The Friedewald formula (FF) is an estimation of LDL-c level that uses the following levels of total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-c): $LDL-c (mg/dL) = TC (mg/dL) - HDL-c (mg/dL) - TG (mg/dL)/5$.

Tandem mass spectrometric analysis of methylarginines

Serum methylarginine and related metabolite levels (ADMA, SDMA, LNMMA, arginine, ornithine, citrulline and homoarginine) were restrained with the validated technique developed by Eryavuz Onmaz *et al.*⁹. Briefly, 200 µL of plasma specimen, 100 µL of ADMA inner norm (d7-ADMA in MeOH), 1000 µL of methanol were taken into eppendorf tubes. This mixture was vortexed for 30 seconds and centrifugated at 13000 rpm for 10 min. The supernatants were given in glazing reaction pipes and evaporated under nitrogen gas at 60°C. For the derivatization process, 200 µL of butanol solution consisting of 5% (v/v) acetyl chloride was added. The sealed pipes were incubated for 30 min at 60°C. After derivatization process, the mixtures were evaporated with nitrogen gas. 200 µL of water-methanol (90:10, v/v%) mixture including 0.1% (v/v) formic acid was added to dissolved the residues and 40 µL mixture was injected into the tandem mass spectrometry system.

Chromatographic separation was performed with Phenomenex C18 HPLC column (50 mm × 4.6 mm) and the Shimadzu HPLC system (Kyoto, Japan) consisted of a pump (LC-20 AD), an automatic sampler (SIL-20 AC HT), and a unit for online degassing (DGU-20A3). Mass spectrometric detection was carried out working an API 3200 triple quadrupole mass spectrometer (Applied Biosystems/MDS Sciex) equipped with an electrospray ion source (ESI) operating in positive format. The moving phases A and B consisted of 0.1% formic acid in water, and 0.1% formic acid in methanol, one way one.

Statistical analysis

Statistical analysis was performed with the SPSS statistical software package version 22.0. The distribution of data was determined with the One-

Sample Kolmogorov-Smirnov. The parametric data was compared with the Student's t and Mann-Whitney U test was used for the comparison of the nonparametric data. Spearman's correlation test was used for the correlation analysis. $p < 0.05$ was considered to be statistically significant.

Results

The research included 100 volunteer women aged 20 to 64 years. The demographic data of and the laboratory parameters of the subjects are shown in Table 1 & Table 2, respectively.

As a result of statistical analysis, ADMA ($p=0.009$), SDMA ($p=0.029$), citrulline ($p=0.001$) levels and total methylarginine load ($p=0.008$) were found to be higher in cup blood contrast to venous blood. There was no statistically important divergence between LNMMA, arginine, homoarginine and ornithine levels ($p > 0.05$). The comparison of the methylarginines were expressed in Table 3.

Discussion

Although hi-jama therapy is an old process technique dating back thousands of years, it has now begun to be widely applied in many countries, including the USA, England and Germany, as an alternative and complementary method in the process of long-term illness or conditions that do

unresponsive to traditional treatment¹⁰. However, the mechanism of action of hi-jama method of healing therapy is not yet fully understood.

There are various theories on this subject¹⁰. One of these is the blood detoxification theory. This hypothesis is based on the principle that wet cupping therapy removes toxic substances from the blood. It is thought that cupping therapy removes toxins, harmful metabolites and oxidant compounds whose levels increase from the body. Wet cupping therapy is thought to extract toxins and harmful substances from the blood through the negative pressure suction process created¹¹⁻¹⁵. There are studies comparing various biochemical, hematological and immunological markers into venous blood and cup blood. In this way, it is tried to shed light on the potential mechanism of cupping therapy in various pathologies. Saeed *et al.*¹³ showed increased uric acid, HDL, LDL, glutamic oxaloacetic transaminase and iron levels in cup blood. Rahman *et al.*¹² reported that hi-jama reduces cholesterol, triglyceride, low-density lipoprotein, fasting blood sugar, ferritin, urea and creatinine levels in patients and lowers blood pressure. Saeed *et al.*¹⁵ similarly reported increased levels of cholesterol, triglycerides, as well as low-density lipoproteins (LDL), high-density lipoproteins (HDL), glucose, uric acid, and urea in cupping blood relative to venous blood. Tagil *et al.*¹⁴ reported that MPO reduces oxidative stress markers, MDA and NO levels were higher and SOD activity was lower in hi-jama blood than in venous blood in simultaneously taken cup blood samples. Eryavuz *et al.*⁶ compared oxidant/antioxidant markers in venous blood specimens taken before and after the application from participants who underwent wet cupping therapy for 3 months. It was reported that the highest MDA and TOS levels, and the lowest TAS, GSH, SOD and CAT levels were detected in the blood samples was taken before the study¹⁵. Similar to these studies, our team has shown that oxidative stress-related miRNAs

Table 1 — Demographic data of the participants.

Parameters	Subjects (n=100)
Age (years)	47.0 (20-64)
Gender (n, %)	Female (100, %100)
Education (n, %)	Primary (15, 15%) Secondary (55, 55%) University (30, 30%)
Length (cm)	1.61 (1.50-1.80)
Weight (kg)	68 (45-89)
BMI (kg/m ²)	25.5 (18-30)

BMI: body mass index.

Table 2 — Biochemical findings of the participants

Biochemical parameters	Participants (n=100)
Glucose (mg/dL)	100.3±15.1
Cholesterol (mg/dL)	205.2±34.5
LDL (mg/dL)	117.7±28.9
HDL (mg/dL)	52.5±11.2
VLDL (mg/dL)	29.8±13.6
TG (mg/dL)	151.3±67.8
Urea (mg/dL)	28.7±8.7
Creatinine (mg/dL)	0.78±0.08
ALT (IU/L)	17.9±8.4
AST (IU/L)	17.5±4.6
Hemoglobin (g/dL)	13.1±1.1

Table 3 — Comparison of the serum methylarginine derivatives.

	Venous blood	Cupping blood	p
ADMA (µM)	0.14 (0.06-0.25)	0.16 (0.07-0.30)	0.009
SDMA (µM)	0.15 (0.02-0.25)	0.16 (0.06-0.33)	0.029
LNMMA (µM)	0.16 (0.01-0.25)	0.17 (0.01-0.30)	0.559
Arginine (µM)	35.9 (8.4-122.8)	44.8 (8.2-101.0)	0.071
Citrulline (µM)	47.1 (11.9-120.0)	65.2 (23.7-174.0)	0.001
Ornithine (µM)	27.9 (5.7-103.0)	25.7 (5.7-125.0)	0.897
Homoarginine (µM)	1.62 (0.50-3.08)	1.41 (0.26-25.1)	0.926
Total methylated arginine load	0.31 (0.13-0.46)	0.34 (0.15-0.65)	0.008

miRNA-34a, miRNA-21 and miRNA-200a levels and serum MDA levels are higher in cup blood than in venous blood, while GSH levels are lower⁶. In addition, there are different studies showing that wet cupping therapy remove the heavy metals from microcirculation. Umar *et al.*¹⁶ reported that reduced blood heavy metals after 30 days cupping therapy. Gok *et al.*¹⁷ showed that aluminum, lead, silver and mercury levels in wet cupping blood were remarkably higher than in venous blood samples. Our findings showed increased ADMA, SDMA, citrulline levels and methylarginine load in wet cup blood. Although LNMMA levels were higher in cup blood than in venous blood, they were not statistically significant. Studies have shown that elevated serum ADMA levels are related with cardiovascular and metabolic illness such as hypercholesterolemia, hypertension, chronic heart failure, stroke, and diabetes, and that ADMA is an liberated risk factor for cardiovascular diseases¹⁸. Additionally, increased serum ADMA levels have been detected in inflammatory and autoimmune disorders such as ankylosing spondylitis, rheumatoid arthritis, gout, and cerebrovascular pathologies such as multiple sclerosis and stroke¹⁹⁻²¹. Therefore, increased methylarginine load is considered a risk factor for cardiovascular, renal, cerebrovascular and autoimmune diseases. The fact that the excretion of methylarginine in wet cup blood is relatively higher than that of arginine in venous blood strengthens the hypothesis that wet cup therapy can play a complementary role in the protection and treatment of these diseases through the detoxification of harmful metabolites from the blood.

Methylarginines generally show these effects by reducing endothelial NO release. NO mediates vasodilation, blood flow and blood pressure regulation. It is a signaling gas that has immunomodulatory effects and is involved in the process of neurotransmission and cell differentiation²². Therefore, NO mediates important biological roles. It is thought that one of the potential mechanisms of cupping therapy may be the triggering of some biologically beneficial changes due to the release of NO from endothelial cells. In an experimental study, it was reported that the expression of the NO synthase enzyme, which catalyzes the production of NO from L-arginine, increased round the cutis acupuncture points of rats²³. It has been proven by numerous studies that cupping current capillaries expands and increases dermal

blood circulation. Blood vessels in the areas where cupping is applied expand with the free of vasodilators such as adenosine, noradrenaline and histamine, thus increasing blood circulation²⁴. Tagil *et al.*¹⁴ showed higher nitric oxide levels in cupping blood measured to venous blood. The increased release of NO from endothelial cells due to cupping therapy root vasodilation, decrease in vascular resistance, decrease in blood stress, hinder of platelet aggregation and adhesion, inhibition of leukocyte adhesion and migration, and decrease in flat beef multiplication. It is thought that it may prevent the formation of atherosclerotic plaques. It is thought that one of the potential mechanisms that hi-jama treatment may be effective in increasing NO release may be the increase in the expression of the nitric oxide synthase enzyme in the application area²⁵. However, considering our findings, we also think that hi-jama treatment may lead to increased NO levels by increasing the excretion of methylarginines.

Conclusions

This studies showed that ADMA, SDMA and citrulline levels were significantly higher in cup blood than in venous blood. Total methylarginine burden was higher in cup blood than in venous blood. Therefore, our findings revealed that cupping therapy may act through the detoxification of harmful metabolites from the blood. Biochemical studies are needed, especially with blood samples taken from cupping applied to important points of acupuncture, to see how it regulates many homeostatic regulations, apart from just removing dirty blood from the body, and how many other systems are regulated when toxic loads are removed from the systems.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

FHY and HA conceptualization; formal analysis; funding acquisition. coordinated the study, enrolled the patients and performed the follow-up visits. BU and DEO resources; software; supervision; roles/writing - original draft; writing - review & editing.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and research committee and with the 1964 Helsinki Declaration. The study was approved by local ethics committee (Selcuk University ethics committee (Number: 2022/172, Date: 29/03/2022). All participants gave informed written consent.

Data Availability

All data underlying the study may be available from the corresponding author upon reasonable request.

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