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A comparison of the some components in blood before and after cupping therapy

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Abstract
Cupping therapy (Hejamat) is a traditional medical therapeutic procedure that is still practiced in many societies today. This study aimed to examine the biochemical, immunological, and hematological features of cupping and venous blood The goal of the current study was to determine how cupping therapy affected the levels of serum lipids. and biochemical, immunological, and hematological parameters to be compared. Fifty men (30 to 50 years old), who had hyperlipidemia but had not taken antihyperlipidemic medication or consumed a high-energy diet during the research were cupping. Each volunteer provided a 16 mL venous blood sample at the start of the trial. The volunteers were subsequently given cupping therapy and determined before cupping and then for two weeks after cupping. The Selectra auto analyzer was used to assess biochemical variables; the KX21 cell counter was used to assess hematological components; and the Western green method was used to assess sedimentation rate. A sensitive sandwich ELISA kit was used to measure cytokines. While there were no significant variations in the serum concentrations of HDL cholesterol and triglycerides, patients with hyperlipidemia who underwent cupping showed a significantly increased ($p \leq 0.001$) level of total cholesterol and LDL cholesterol in comparison to before cupping. The results indicated that some changes had taken place in the instances following cupping, leading us to conclude that this pain relief was only temporary and that cupping should not be used as the sole form of treatment. However, if done improperly, cupping can have adverse effects.

Keywords: Biochemical factors, hematological factors, immunologic-response, cupping, venous cupping

1. Introduction
One of the most well-documented medical techniques is cupping therapy [1] {Kh, 2001 #4}. It's a centuries-old method of diagnosing and treating a wide range of illnesses. Eber's papyrus from Ancient Egypt (1550 BC) was one of the first pieces of medical literature that discussed cupping therapy [2]. Cupping therapy is used in Unani, Tibetan, Chinese, traditional Korean, and Oriental medicine, among other historic healing systems [3]. Hippocrates, an ancient Greek physician, published lengthy descriptions of cupping applications. Wet cupping and dry cupping are the two subtypes of cupping therapy. While wet cupping pushes the suctioning cup with the local underlying tissue similarly to dry cupping, it also involves scarification and bloodletting [4]. It is an old therapy for treating and curing a wide range of ailments, including blood diseases, hypertension, rheumatic conditions, pain alleviation, inflammatory conditions, physical and mental relaxation and stress [5], Menopause syndrome [6], hemochromatosis, polycythemia [7], hyperlipidemia [8], discomfort of the knee, liver disorders, renal and ureteric colic, and other problems [2]. Cupping's purpose is to eliminate potentially harmful blood from the body, so relieving the body of potential harm from symptoms and resulting in a drop in well [9]. Cupping therapy, also known as Al-Hejamah in Arabic, has been a staple of Middle Eastern cultural practice for thousands of years, with mentions reaching back to Hippocrates' (400BC) period [10]. Cupping is taught as a part of complementary medicine (CM) in 60% of medical schools in the United States [11]. It's also used at Johns Hopkins Hospital and Harvard Medical School, two of the world's most prominent medical institutions [12]. Although our sources are about traditional and complementary medicine, including cupping, and despite the progress of science in some areas, our information in this field, based on traditional experiences and

references to texts left over from the past, is solid. With scientific justification and determining the mechanisms of biological actions, cupping can be used more correctly and more reliably [10].

Cupping is viewed with greater skepticism than other traditional medical approaches have. Although cupping is methodical, there has been a resurgence of interest in traditional medicine in recent years. Different viewpoints from both scientific and non-scientific authorities and groups have been expressed regarding cupping. Since a group of adversaries felt that cupping was a form of bleeding, it is outdated that with the availability of intravenous bleeding in modern medicine, the practice is no longer necessary [8]. The results of the current investigation described the biochemical, hematological, and immune reactions to cupping.

2. Materials and methods

2.1. Subjects

Volunteers were men aged 30–60 years from Cupping Center, Al-Suwaira district, Wasit Governorate, Iraq, they were chosen at random for the current study after being clinically and pathologically diagnosed as hyperlipidemic patients by a professional practitioner. The sampling was done in 2-4 hours during the afternoon from January to April 2021. None of the participants had a history of abortion. They did not have a chronic illness.

2.2. Sample collection

For intravenous blood sampling, 16 ml from each volunteer liter of venous blood before and after cupping and then for two weeks after cupping were taken as follows: Ten milliliters of blood were drawn using tubes without anticoagulant in order to prepare the serum for biochemical factor testing. For CBC, WBC types, and sedimentation testing, four milliliters of venous blood were drawn using K_3EDTA anticoagulant tubes. Tubes containing one milliliter of venous blood per tube from the patient were used for PHA mitogen culture and mitogen-free culture media for immunological tests. The Australian company Cellestis produced this material.

2.3. Measurement of parameters

The individual was prepared for cupping in the following way after venous blood sampling: The cupping position was cleaned with alcohol on T5-T2 beads, and this area was then rinsed with acetone to ensure that any skin fat present would not obstruct the measurement of fat levels. Next, we used cupping glasses to set the position for 5 minutes and used sterile razor blades with the deepest possible grooves to cut the skin. Three blood samples are typically taken during each cupping. Complete the cupping process, then bandage the cupping location.

As soon as possible, blood was drawn for biochemical tests, centrifuged for 10 minutes at 3000 rpm at 6 °C, and the serum was then stored in the freezer for 70 hours. Following sample collection utilizing the Biochemical Systems Diagnostic kits and Selectra autoanalyzer from the German company Merk. Using an enzyme kit from the French company Biolabo, total cholesterol, HDL triglycerides, and cholesterol levels in the blood were measured [13]. The Friedwald formula was used to determine the indirect serum LDL: [Total cholesterol - (HDL+TG/2.2)] [14].

To evaluate the immunology of tubes containing milliliters of mitogenic PHA and mitogen-free traction medium immediately, blood was poured into a moving, 37°C incubator. Following 16 to 24 hours in the incubator, the tubes were taken out and centrifuged at 2500 rpm for 15 minutes, after which the culture supernatant and sample were collected, and so on until the samples were finished and stored in the freezer. Interleukin-4 and interferon-gamma levels were evaluated using an ELISA D&R kit after the entire sample was collected.

A 21-KX selector made in Japan was used to evaluate the hematological factors. Burn staining 3.5 mm was used to induce staining in the appropriate area [6, 15].

In venous cupping using EDTA-specific glasses In order to examine immunology and remove hematology, six milliliters of cupping blood were stained in the same manner as venous blood samples. Then, a second stage of bleeding was performed using glass for cupping without an anticoagulant. Ten milliliters of blood were needed at this stage for measurement. By using the Wester Green method, sedimentation was manually measured.

2.4. Stastical method

The obtained results were entered into the statistical program SPSS. The biochemical, hematological, and sedimentation factors were examined using independent tests and t-tests, while immunological and other factors were examined using the nonparametric Wilcoxon test. When p <0.001, the significant level was taken into account [16].

3. Result and Discussion

The results show in Table 1 that patients with hyperlipidemic who subjected to cupping show a significant increase ($p \leq 0.001$) in total cholesterol, LDL cholesterol in weeks two after cupping by comparison before cupping. While there were no significant changes in serum HDL cholesterol and triglyceride TG in weeks two by comparison before Cupping ($p \geq 0.001$). While uric acid and alkaline phosphatase (ALK) were less in weeks two after cupping by comparison before cupping ($P \leq 0.001$), while other parameters like glutamyl oxalate transaminase (SGOT), C-reactive protein (CRP) and iron were larger in in weeks two after cupping by comparison before cupping ($P \leq 0.001$). While that erythrocyte sedimentation rate (ESR was less in weeks two after cupping by comparison before cupping and statistically non-significant (p=0.013) (This decrease may be due to an increase in the number red cells per unit volume and consequently a decrease in the total amount of proteins [12].

In Table 2 the results show that hemoglobin and mean corpuscular hemoglobin concentration (MCHC) were more statistically significant ($P \leq 0.001$) in weeks two after cupping by comparison before cupping, while the platelets and MCV were less statistically significant weeks two after cupping by comparison before cupping ($P \leq 0.001$). Additionally, the results show that there are more red blood cells, white blood cells, hematocrit, and viscosity weeks two after cupping by comparison before cupping and that this difference is statistically non-significant ($P \geq 0.001$).

The non-parametric Wilcoxon test was performed to compare the WBC type levels before and after two weeks cupping. Results indicated the level of neutrophils, monocytes, and eosinophils was lower weeks two after cupping by comparison before cupping and statistically non-

significant ($P \geq 0.001$), but the number of lymphocytes in the cupping blood after two weeks was higher and statistically significant ($P \leq 0.001$). This increase in the percentage of lymphocytes may be related to lymph and interstitial fluid being drawn along with cupping blood. Table 2 presents the outcomes.

By applying the nonparametric Wilcoxon test, the immunological characteristics. These findings demonstrated that the stimulus index is obtained differences in response to mitogen PHA stimulation and the response is in the unstimulated state (basal state). The level of interleukin-4 was more statistically significant in weeks two after cupping by comparison before cupping ($P \leq 0.001$), while interferon-gamma was less than in weeks two after cupping by comparison before cupping and statistically non-significant ($P \geq 0.001$), and this may suggest that the blood in the cupping is faced with stimulation, and it has less response power. Table 3 displays the outcomes.

Despite the fact that contemporary medicine has undergone numerous improvements in the medical and scientific sciences, many people around the world still pay attention to other treatments such as herbal medicine, homeopathy, acupuncture, energy therapy, cupping, etc. The frequency of use of these methods, which are called complementary or alternative medicine (complementary or alternative medicine), is increasing all over the world [11]. Since several medical supplementing techniques have been shown via scientific research to be helpful and safe in the treatment of specific diseases, they are now used as integrated medicine techniques [17, 18].

Despite the lack of a clear mechanism of action and insufficient data to support their efficacy in comparison to modern therapy, many patients continued to use cupping. As mentioned in the introduction, cupping is one of the methods of therapy that is a traditional medicine that has been around since ancient times. Most countries use it in various forms. Historical records have shown that people from different nations used cupping for disease treatment and prevention [4, 9]. Because some individuals think cupping is an outdated form of blood sampling due to the availability of intravenous blood sampling, the authors felt the need to compare venous blood with blood from cupping [19, 20]. The present study investigates the hypothesis that the biochemical composition, hematology, and immunology of venous blood and blood from cupping are different. The results of this study on the biochemical composition of venous blood and blood from cupping showed that uric acid, iron, TG, LDL, HDL and SGOT, in cupping blood significance is higher than the amount of these factors in venous blood. On the other hand, the amount of ALK in the blood of the cupping significance is lower than the amount of this factor in venous blood. This result was compatible with research conducted in Syria on RBCs, which are excreted through the blood of the cupping they take themselves [2].

Results obtained from the difference between venous blood and blood obtained from cupping in terms of hematology and sedimentation factors showed that the amount of RBC, Hb, hematocrit, and viscosity in the blood cupping is significantly larger than the venous blood volume. The ESR in the blood from cupping is lower than in the venous blood. This decrease can be due to an increase in the number of cells per unit volume and, consequently, a decrease in the total amount of protein [12, 21].

To study the immunological composition of venous blood and resulting blood cupping, first one milliliter of venous blood and one milliliter of blood cupping in culture medium containing PHA mitogen and environment culture without culture were cultured. Then, using the method ELISA, levels of interferon-gamma and interleukin-4 are measured, respectively. The response patterns of Th1 and Th2 are measured. Then the excitatory index of the difference between the basal interferon concentration gamma and interleukin 4 and concentrations in the stimulated state with mitogen of both was calculated. The data obtained indicated this. The stimulus index in cupping blood is significantly lower. It is from a blood vessel. That is, the blood from cupping in the face of strong stimulation of mitogen will not be able to respond. This matter may indicate that the lymphocytes that through cupping are excreted, normal function and function do not exist.

The results obtained in this study were compatible with the results obtained from a comparison of the biochemical composition of blood and blood from cupping by Dr. Agin, which was performed in 1993 [3, 22]. And also, the results obtained from the comparison of the biochemical composition of venous blood and blood from cupping by Dr. Montazer, which was conducted in 1998, were compatible with our results [14].

In the only study abroad in Syria in 2001 by Allameh Mohammad Amin Sheikho *et al*., cupping was performed [2, 23]. This study also confirms our results. A study compared some biochemical and hematological factors in the blood from cupping and venous blood treatments, and the results have been collected in the book "Dawa Al-Ajib." What are the objections in some circles?

Cupping is done in the same way as cupping, also known as intravenous blood sampling. But according to the above results, venous blood sampling and cupping blood sampling were combined. They are different. This difference can be due to the position of cupping or due to the method of blood sampling by cupping. In traditional medicine books for cupping, they have mentioned different positions and the effects of each position. They mentioned special treatment [4, 9, 24]. This difference in the places of blood sampling is one of the important factors in the difference in intravenous blood and blood from cupping [3]. Laying down and causing congestion and inflammation is an integral part of cupping [13]. For example, it is possible to mention important effects for retrieval alone. Blowing, in addition to causing cell recall safety and inflammation in the position of cupping can occur, blood also helps areas that need treatment [10, 14].

Venous blood and cupping blood differ in their components and immune responses, according to the comparison between the two types of blood. Based on the above evidence, it can be claimed that cupping is not a simple blood sampling method and, based on documented evidence, it is a method of treatment in the books of traditional medicine and Islamic medicine [4, 9].

Table 1: Comparison of biochemical composition of venous blood and blood from cupping

Factor	Blood collection	No.	Mean± SD	p-value
Uric acid	Before cupping	50	20.1±1.01	0.000*
	After 2 week from cupping	50	7.77±1.52	
Cholesterol	Before cupping	50	201.11±22.02	0.000*
	After 2 week from cupping	50	225.27±14.22	
Triglycerides	Before cupping	50	156.10±84.35	0.000*
	After 2 week from cupping	50	170.11±74.31	
LDL	Before cupping	50	98.54±30.17	0.000*
	After 2 week from cupping	50	100.08±1.72	
HDL	Before cupping	50	63.51±2.42	0.016
	After 2 week from cupping	50	55.71±1.47	
SGOT	Before cupping	50	25.44±11.10	0.000*
	After 2 week from cupping	50	36.50±17.30	
ALK	Before cupping	50	230.31±85.72	0.000*
	After 2 week from cupping	50	163.60±91.05	
CRP	Before cupping	50	1.62±1.08	0.000*
	After 2 week from cupping	50	1.81±1.21	
Iron	Before cupping	50	95.35±31.34	0.000*
	After 2 week from cupping	50	156.37±16.42	
ESR	Before cupping	50	4.41±1.42	0.015
	After 2 week from cupping	50	2.60±0.81	

LDL; low density lipoprotein alkaline phosphatase, HDL; high density lipoprotein, SGOT; glutamyl oxalate transaminase, ALK; alkaline phosphatase, CPR; C-reactive protein. ESR; erythrocyte semination rate, (*) significant (P<0.001 (*).

Table 2: Comparison of the level of hematological factors in venous blood and blood from cupping

Factor	Blood collection	No.	Mean ±SD	p-value
WBC	Before cupping	50	3122.08±1640.18	0.141
	After 2 week from cupping	50	5698.39±1014.38	
RBC	Before cupping	50	5.42±0.37	0.004
	After 2 week from cupping	50	5.63±0.57	
Hb	Before cupping	50	15.15±1.2	0.001*
	After 2 week from cupping	50	16.49±2.4	
Hematocrit	Before cupping	50	43.93±2.68	0.002
	After 2 week from cupping	50	46.57±5.56	
Blood viscosity	Before cupping	50	3.14±0.09	0.002
	After 2 week from cupping	50	3.25±0.21	
MCV	Before cupping	50	93.84±4.25	0.044
	After 2 week from cupping	50	92.67±5.38	
MCH	Before cupping	50	38.91±2.22	0.036
	After 2 week from cupping	50	39.13±2.29	
MCHC	Before cupping	50	35.65±1.51	0.001*
	After 2 week from cupping	50	36.99±1.85	
Platelets	Before cupping	50	212.17±50.23	0.001*
	After 2 week from cupping	50	116.51±62.06	
Neutrophils	Before cupping	50	62.66±11.27	0.005
	After 2 week from cupping	50	57.92±10.26	
Lymphocytes	Before cupping	50	34.70±10.06	0.001*
	After 2 week from cupping	50	60.92±10.03	
Monocytes	Before cupping	50	1.36±0.33	0.006
	After 2 week from cupping	50	0.86±0.71	
Eosinophil	Before cupping	50	1.62±1.09	0.042
	After 2 week from cupping	50	0.56±0.15	

WBC; white blood cell, RBC; red blood cell, Hb; hemoglobin, MCV; mean corpuscular volume, MCH; mean corpuscular, MCHC; mean corpuscular hemoglobin concentration, (*) significant (P<0.001)

Table 3: Comparison of the stimulus index of Th2 and Th1 responses in venous blood and blood from cupping.

Factor	Blood collection	No.	Mean± SD	p-value
Stimulus index of interferon-gamma	Before cupping	50	8150.04±3185.6	0.002
	After 2 week from cupping	50	4293.50±1561.4	
Stimulus index of interleukin 4	Before cupping	50	323.45±184.41	0.001*
	After 2 week from cupping	50	544.24±112.38	

(*) significant (P<0.001)

4. Conclusion: Despite the precise mechanism of cupping therapy, the finding of the present study do not appear to allow for the determination of its therapeutic effects. Instead, they show that cupping therapy is significantly

correlated with changes in inflammatory levels and dissolved markers, suggesting that it may affect the patient's inflammatory state and improve clinical outcomes.

The biochemical and immunological composition of cupping blood is such that even if this blood can be collected, for the sake of its high concentrations of excreta, it is not usable for people in need. The last word is that the mechanism of cupping and its therapeutic effects are unknown and still need comprehensive research, so it is impossible to comment on its potential benefits and harms. Are there differences in the combination of immunology, biochemistry, and hematology? Is venous blood and blood from cupping beneficial to the physiology of the human body? And can this difference lead to physiological changes in the body? To be? To achieve these goals, it is suggested that changes in biochemical, immunological, and hematological parameters be made after cupping.

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6. Data availability

The data generated and analyzed in the presented study are available from the corresponding author on request.

7. Conflicts of Interest

The authors have stated that they have no conflicts of interest.

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