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Evaluation of the Effects of Traditional Cupping on the Biochemical, Hematological and Immunological Factors of Human Venous Blood

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1. Introduction

1.1 Traditional medicine

Since 30 yr ago, the World Health Organization (WHO) has considered the development of Traditional Medicine (TM) in order to implement the slogan "Health for all by the year 2000 A.D.". The decision was based on two foundations; first, lack of access of a great number of people (up to 80% in some countries) to primary healthcare and second, dissatisfaction from the outcomes of treatments by modern medicine, especially in relation to chronic diseases and the side effects of chemical drugs (WHO, 2002). In 2002 AD, WHO has described traditional medicine in more details as: "Traditional medicine is a comprehensive term used to refer both to TM systems such as traditional Chinese medicine, Indian Ayurveda and Arabic-Unani medicine, and to various forms of indigenous medicine. Traditional medicine therapies include medication therapies if they involve use of herbal medicines, animal parts and/or minerals and non-medication therapies if they are carried out primarily without the use of medication, as in the case of acupuncture, manual therapies and spiritual therapies (Lozoya, 1994; WHO, 2002).

The comprehensiveness of the term "traditional medicine" and the wide range of practices it encompasses make it difficult to define or describe, especially in a global context. Traditional medical knowledge may be passed on orally from generation to generation, in some cases with families specializing in specific treatments, or it may be taught in officially recognized universities. Sometimes its practice is quite restricted geographically, and it may also be found in diverse regions of the world. However, in most cases, a medical system is called "traditional" when it is practiced within the country of origin (Bodeker & Kronenberg, 2002).

1.1.1 The situation in the use of traditional and complementary /alternative medicine

Traditional and complementary/alternative medicine is widely used in the prevention, diagnosis, and treatment of an extensive range of ailments. There are numerous factors that have led to the widespread and increasing appeal of traditional and

complementary/alternative medicine throughout the world, particularly in the past 20 years. In some regions, traditional and complementary/alternative medicine is more accessible. In fact, one-third of the world's population and over half of the populations of the poorest parts of Asia and Africa do not have regular access to essential drugs. However, the most commonly reported reasons for using traditional and complementary/alternative medicine are that it is more affordable; more closely corresponds to the patient's ideology, and is less paternalistic than allopathic medicine. Regardless of why an individual uses it, traditional and complementary/ alternative medicine provides an important health care service to persons both with and without geographic or financial access to allopathic medicine. Traditional and complementary/alternative medicine has demonstrated efficacy in areas such as mental health, disease prevention, treatment of non-communicable diseases, and improvement of the quality of life for persons living with chronic diseases as well as for the ageing population. Although further research, clinical trials, and evaluations are needed, traditional and complementary/alternative medicine has shown great potential to meet a broad spectrum of health care needs (Shaikh & Hatcher, 2005).

1.1.2 Traditional medicine in Iran

The practice and study of medicine in Persia has a long and prolific history (Madari & Jacobs, 2004; Miraldi, Ferri, & Mostaghimi, 2001). In recent years, some experimental studies have indeed evaluated Medieval Iranian medical remedies using modern scientific methods. These studies raised the possibility of revival of traditional treatments on the basis of evidence-based medicine (Gorji & Ghadiri, 2002). Iranian traditional medicine is as a vivid and perfect ideology that behold the world as the best order and creation of the Lord, and has named physician because of his/her mastery and encirclement in different sciences beside of exact hell of him as philosopher (Danyali, Mahdavi, Ghazanfari, & Naseri, 2009). In this paradigm which gives priority to human's life style, fourfold phlegm theory including blood, phlegm, yellow bile and black bile is began according to fourfold ingredients like fire, air, water and dust; and the equilibrium between these gradients causes well-being (Elgood, 2010). The medieval medical texts of Persia such as *Qanoon-fel-teb* (The Canon) by Ebn-e-Sina, Râzi's *Ketab-al-hawi*, and *Zakhireh Kharazmshahi* by Esmail Jorjani became the most widely read literatures in medieval Europe. During renaissance, medieval Persia had a significant influence on the contemporary medicine; this influence is still felt today. In recent years, some experimental studies have evaluated medieval Persian natural therapies using modern scientific methods. These investigations raised the possibility of revival of traditional treatments (Ghadiri & Gorji, 2004).

Traditional Iranian Medicine (TIM) roots back to over 8000 yr B.C. and it is a branch if not the root of the so called Arabic-Unani medicine as quoted by Cyril Elgood, the English medical historian, was more advanced than that of Assyria and it is not too bold to go even further and claim that this school of medicine was created firstly in ancient Persia and then transferred to Greek and consequently during Islamic-Arabic civilization developed all over the world; so that this school can be named as Iranian-Unani or Arabic-Unani medicine which has been known ever since as Greek medicine (Rezaeizadeh, Alizadeh, Naseri, & Ardakani, 2009).

Today, as more people seek alternative therapies to deal with their health problems, the use of traditional medicine, including cupping is increasing. Cupping is considered as one of the curative skills in TIM that was used for many years all over the world. Despite there are lots

of resources about traditional medicine especially hijama; our knowledge in this regard is insufficient and is just on the basis of referring to previous literatures. So it seems by identifying all scientific aspects of traditional medicine like its mechanisms, we can use it easily and introduce it to modern medicine with a powerful credit.

1.2 Cupping history

Although there is reason to believe the practice dates from as early as 3000 B.C., the earliest record of cupping is in Ebers Papyrus, one of the oldest medical textbooks in the world. It describes in 1,550 B.C. Egyptians used cupping. Archaeologists have found evidence in China of cupping dating back to 1,000 B.C. In ancient Greece, Hippocrates (c. 400 B.C.) used cupping for internal disease and structural problems. This method in multiple forms spread into medicine in Asian and European civilizations.

Cupping in Europe and the Middle East grew from humoral medicine, a system of health ancient Greeks used to restore balance through the four "humors" in the body: blood, phlegm, yellow bile and black bile (Sweet, 1999).

Way back in time, long before any historical or archeological evidence had been uncovered to support the application of cupping instruments to the body as a therapeutic procedure, prehistoric humans relied in part on their ability to suck and draw to the surface any irritations such as stings and thorns. Early humans also developed conceptualizations concerning their place in nature and the universe and the causes of ill health.

In their efforts to explain sickness, they held beliefs about that which could enter the body or mind such as evil spirits and cause pain and suffering. Many researchers including anthropologists have described how healers of these super naturalistic traditions of illness causation applied oral suction to the surface of the body to withdraw the effects of these malevolent influences (Jackson, 2001; Sagi, Ben-Meir, & Bibi, 1988; Xie, Asquith, & Kivipelto, 1996).

In time, various natural resources began to be used to effect suction - which makes good sense because indigenous groups could exploit their natural resources. For example, natives along the west coast of North America, in the vicinity of Vancouver Island, used shells. In Europe, Asia, Africa and North America, hollow animal horns were fashioned to provide an effective cupping device. In North America, the natives made their cupping implements by slicing off the point of a buffalo horn. They would then place the base of the horn on the body and suck the air out through the opening at the tip. When a vacuum was achieved, a wad of dried grass would be immediately thrust into the opening by the nimble workings of the tongue. By this method the medicine men, with their powerful facial muscles and considerable agility, can make a very successful job of cupping (Skinner, 1995). Another technique used to withdraw disease was by sucking through a bone tube. During the Babylon - Assyrian Empire (stretching from Iraq to the Mediterranean) massage was practiced as well as 'cupping by sucking, with the mouth or by using a buffalo horn' (Hamilton, 2002).

The earliest pictorial record which has been found on cupping is in a carved relief of medical instruments used by the Egyptians around 1500 BC. Cupping vessels of contemporary design can be recognized in the lower corner (Sandhu, 2004).

Textual evidence on cupping can be found in the writings of Hippocrates (C.460-377 BC), known as the father of modern medicine. During this golden era of the early Greek state, Hippocrates and his followers were devoted to an empiric approach to healing and sought naturalistic explanations why people became ill. They thoroughly rejected causes like spirits or ghosts, and instead reasoned that poor diet, insufficient exercise, exposure to adverse weather, an unbalanced lifestyle and emotional factors were the chief agents of ill health. In his guide to Clinical Treatment, Hippocrates recommended cupping for the treatment of angina, menstrual and other disorders (Vaskilampi & Hanninen, 1982).

Hippocrates also wrote about two forms of cupping. These are known as dry cupping and wet or moist cupping. Dry cupping pulls the local underlying tissue up into a cupping vessel, whereas in wet cupping, the skin and sometimes deeper is cut to produce a flow of blood and then a cup is applied. The strong drawing action of the cup increases the volume of blood taken. Though he did practice wet cupping; it seems likely that Hippocrates advocated dry cupping because it was a gentler and safer technique (Bhikha, 2008; Shervani, AnsariI, Shabnam, & Nikhat, 2010)

Cupping remained a constant in professional medical treatment throughout Europe. It was practiced by such famous physicians as Galen (131-200AD), Paracelsus (1493-1541) and Ambroise Pare (1509-90). Cupping was also practiced by other practitioners including barber surgeons and bath house attendants (Chirali, 1999).

Beside, in Iran cupping has a long term history. Avicenna at his book "Qanoon" has mentioned hijama as a medical pillar to management of over 37 kinds of diseases (Dearlove, Verguei, Birkin, & Latham, 1981).

Usamah bin Shuraik narrated that the Prophet said; O servants of Allah! Seek treatment, Allah did not create a disease without creating a cure therefore, except one disease (which is) death.

It is reported that the Messenger of Allah said, "Indeed the best of remedies you have is cupping (hijama) and if there was something excellent to be used as a remedy then it is cupping (hijama)" (Al Jauziyah & Abdullah, 2003).

1.3 What is hijama?

The Arabic word "*hijama*" means "sucking." In the Persian Gulf, *Hijama* was used not only for treatment but also for prophylaxis against diseases. The pearl divers in the Persian Gulf used to undergo *hijama* before the diving season in the belief that the procedure will prevent diseases during the 3 months at sea. It was thought to be very effective against dizziness (Reflections, 2004). Cupping refers to an ancient Chinese practice in which a cup is applied to the skin and the pressure in the cup is reduced (by using change in heat or by suctioning out air), so that the skin and superficial muscle layer is drawn into and held in the cup. In some cases, the cup may be moved while the suction of skin is active, causing a regional pulling of the skin and muscle (the technique is called gliding cupping).

This treatment has some relation to certain massage techniques, such as the rapid skin pinching along the back that is an important aspect of *tuina*. In this practice, the skin is pinched sometimes at specific points (e.g., bladder meridian points), until a redness is

generated (Nielsen, 1995). Common sites for cupping were the temples, behind the ears, the base of the spine and over the upper part of the back. The cups were domed, usually made of glass, and the technique involved the vacuum principle. Tissue paper, rag or alcohol was burned in the cup, which was then flipped over and applied to the skin. The vacuum sucked both tissue and blood into the cup. Later improvements involved using a valve and a pump, eliminating the heating mechanism (which often led to painful blistering). Wet cupping involved scaril fying (developed in the 17th century), the skin with a scarification which had from one to 16 blades. The depth of the blade was regulated by a screw, or a spring known as a Spring Fleam or Schnapper. When the cup was applied to the scarified skin, the blood was drawn out through the wounds. Cupping was still used in the early 20th century, but only for drawing blood from poisoned wounds (Curtis, 1981). Cupping is applied to certain acupuncture points (especially on T2-T5 vertebrates), as well as to regions of the body that are affected by pain (where the pain is deeper than the tissues to be pulled). Heating of the cups was the method used to obtain suction: the hot air in the cups has a low density and, as the cups cool with the opening sealed by the skin, the pressure within the cups declines, sucking the skin into it. In this case, the cups are hot and have a stimulating effect something like that of burning moxa wool (Soyuncu, 2009).

Cupping (hijama) is of 2 types:

Dry cupping- This is the process of using a vacuum on different areas of the body in order to gather the blood in that area without incisions (small, light scratches using a razor). Dry massage cupping is similar to dry cupping but olive oil is applied to the skin (before applying the cups) in order to allow easy movement of the cups. 70% of diseases, pains and ailments are due to the blood being unable to reach certain parts of the body. Dry cupping and dry massaging cupping allow the blood to reach these places.

Wet cupping- This is the process of using a vacuum at different points on the body but with incisions in order to remove 'harmful' blood which lies just beneath the surface of the skin (Chirali, 1999; Michalsen, et al., 2009) (It is recommended that wet cupping is only administered by a cupping therapist).

1.4 Contra-indications and precautions to treatment

Cupping Therapy has no major side effects aside from minimal discomfort due to the method of application of skin cuts to the patient. In cases where the patient's pain threshold is low, a local anesthetic can be administered. Also other possible minor side effects that may occur is the feeling of slight light headedness post Cupping Therapy, this again is similar to the sensation one feels after having had blood taken from the doctor, as Cupping Therapy encourages blood flow to the cupped region (hyperemia), one may therefore feel warmer and hotter as a result of vasodilatation taking place and slight sweating may occur. Again this can be attributed to sound scientific rationale and there is no cause for concern.

Pregnant women or menstruating women, cancer (metastatic) patients and patients with bone fractures or muscle spasms are also believed to be contra-indicated. Also, Cupping Therapy cannot be applied to a site of DVT, where there are ulcers, arteries or places where a pulse can be felt (Chirali, 1999).

2. Studies about cupping

Though cupping therapy has been used for thousands of years, there has been no systematic summary of clinical research on it.

A review study by Cao and his colleagues which was included all clinical studies (published between 1959 and 2008, including 73 randomized controlled trials (RCTs), 22 clinical controlled trials, 373 case series, and 82 case reports) on cupping therapy for all kinds of diseases showed wet cupping was used in majority studies, followed by retained cupping, moving cupping, medicinal cupping, etc. 38 studies used combination of two types of cupping therapies. No serious adverse effects were reported in the studies. According to these results, quality and quantity of RCTs on cupping therapy appeared to be improved during the past 50 years, and majority of studies show potential benefit on pain conditions, herpes zoster and other diseases. However, further rigorous designed trials in relevant conditions are warranted to support their use in practice (Cao, et al., 2010).

Soo Lee and his colleagues in order to summarize and critically evaluate the evidence for and against the effectiveness of cupping for stroke rehabilitation compared cupping with acupuncture, electro-acupuncture and warm needling implied there were not enough trials to provide evidence for the effectiveness of cupping for stroke rehabilitation because most of the included trials compared the effects with unproven evidence and were not informative (Lee, Choi, Shin, Han, & Ernst, 2010).

In another trial Iranian researchers investigated the effectiveness of wet cupping therapy for the treatment of nonspecific low back pain and they concluded that "Wet-cupping is associated with greater short- term clinical benefit than usual care and no adverse effects were reported (Farhadi, et al., 2009).

Other researchers investigated the effect of the cupping therapy as a treatment for the carpal tunnel syndrome and found that " Cupping therapy may be effective in relieving the pain and other symptoms related to CTS (carpal tunnel syndrome) (Michalsen, et al., 2009) ".

Moreover, Zhang and colleagues concluded that the therapeutic effect of cupping therapy was satisfactory for gouty arthritis (Zhang, Liu, & He, 2010).

Ranaei-siadat and colleagues for achieving of relation between some blood parameters and wet cupping, selected statistical samples from the young health male (20-27 years old) and tested some blood parameters before cupping and five times after cupping (one time per month). Interestingly their results showed that cupping can only regulate some blood parameters such as Cholesterol, HDL, LDL, and FBS (Ranaei-siadat, et al., 2004).

A study by Bilal and colleagues was aimed to scientifically evaluate the efficacy of the technique used in cupping, i.e. suction and removal of blood by comparing and analyzing the difference between the compositions of blood samples, obtained through cupping technique versus blood drawn intravenously. There was a significant change in almost all parameters tested as compared to the venous blood samples. On the basis of result assumed that there might be some unknown substance present in the blood which is drawn and discarded through cupping and removal of which might be creating a favorable balance between various vital parameters (Bilal, Alam Khan, Ahmed, & Afroz, 2011).

A study by Ahmed and colleagues was carried out in order to evaluate the efficiency of blood-letting cupping therapy in management of rheumatoid arthritis. To sum up they concluded bloodletting cupping combined with conventional medicinal therapy has several advantages. It significantly reduces the laboratory markers of disease activity and it modulates the immune cellular conditions particularly of innate immune response NK cell % and adaptive cellular immune response SIL-2R (Ahmed, Madbouly, Maklad, & Abu-Shady, 2005).

The aim of a study by Niasari and colleagues was to determine if a reduction in serum lipoproteins, especially LDL cholesterol, is a preventive approach against atherosclerosis. Phlebotomy has been a recommended method to reduce serum lipoprotein levels. They found that wet cupping may be an effective method of reducing LDL cholesterol in men and consequently may have a preventive effect against atherosclerosis (Niasari, Kosari, & Ahmadi, 2007).

Considering that cupping is an ancient curative method that is based on no need to drug administration, and because there is not any detailed mechanism about it; so different suggestions in this regard has been introduced. Some believe that it is only a simple blood transferring and by providing modern venous blood transferring techniques in today's life, there is no need to use cupping. In present study we will discuss about some probable mechanisms underlying the traditional cupping, and will also compare biochemical, hematological and some immunological components of normal blood samples with blood obtained via cupping. Moreover, we will report changes of those factors two weeks after cupping in the same group, expressing if cupping blood is equivalent to venous blood samples. Finally, we discuss about what can be the relationship between blood samples of venous and which was gained by cupping.

3. Methods (sample size, study design and participants)

The study was performed on 56 healthy volunteer men aged between 20 and 40 years who were selected randomly. All samples were collected in May and June of 2008 between 2 to 4 o'clock. And there was no history of chronic disease in these volunteers. At the beginning of the study before performing of cupping, 16 ml venous blood sample was obtained from each volunteer as follows:

- 10 ml of venous blood by gel tubes (BD Co., England) which were not soaked with anticoagulation elements in order to get serum samples.
- 4 ml of venous blood by gel tubes (BD Co., England) which were soaked with K3EDTA in order to assay Sedimentation, CBC and diff.
- 1 ml of venous blood by gel tubes (Cellestis Co., Australia) containing culture environment of PHA mitogen in order to assay immunological markers.
- 1 ml of venous blood by gel tubes (Cellestis Co., Australia) containing culture environment with no mitogen in order to assay immunological markers.

After the collection of venous blood, the procedure of cupping was carried out as follows:

A point was selected at the back just between the two scapulas (bounds of T2-T5 vertebrates).Then, after disinfecting of this point by rubbing alcohol, it was ablated with acetone in order to decrease the effect of skin's lard on evaluation of blood cholesterol, LDL, TG, HDL.

Each wet-cupping treatment procedure lasted about 5 min and was conducted in seven steps:

1. *Primary sucking*: The cup was placed on the selected site and the air inside the cup rarified via manual suction. The cup clings to the skin and was left for a period of 5 min.
2. *Scarification*: Superficial incisions are made on the skin using the ''multiple superficial incisions'' technique with sterile surgical blades for incision. In the ''multiple superficial incisions'' technique, we applied multiple superficial incisions. As a result, after healing of the wound, the scar lesion does not remain.
3. *Bloodletting*: The cup which was soaked with EDTA was placed back on the skin, using the same manner described above, until it is filled with blood from the capillary vessels.
4. *Removal*: The cup was removed after 3 min; and from this collected blood, 4 ml was spilled into gel tubes (BD Co., England) which were soaked with K_3EDTA in order to assay Sedimentation, CBC and diff, as well as spilling of 1 ml of this gained blood into both gel tubes (Cellestis Co., Australia) containing culture environment of PHA mitogen or with no mitogen in order to assay immunological markers.
5. The process repeated by using a cup which was not soaked with EDTA in order to evaluate biochemical factors in gained blood of cupping. We collected 10 ml of blood by this process.
6. The cup was placed back again on the skin in order to done all three stages of cupping.
7. *Dressing* (Ahmed, et al., 2005; Niasari, et al., 2007).

3.1 Biochemical testing

Sera were immediately separated out by centrifuging the blood samples at 3000 rpm for 15 minutes; and the parameters including Uric Acid, LDL, HDL, Cholesterol, TG, SGOT, SGPT, CRP and serum Iron were analyzed by Selectra Semi-automatic chemistry analyzer (Merck, Germany), using standard kits supplied by Merck.

3.2 Hematological testing

Hematological parameters were analyzed using automatic Humacount plus, Hematology analyzer (KX-21, Japan). Hematological parameters which were tested include RBC, WBC, hemoglobin, HCT, MCV, MCH,

MCHC, platelets and blood viscosity, in blood samples obtained from veins through standard procedure, and in blood samples obtained through the technique of cupping.

3.3 Sedimentation rate

Sedimentation rate was measured by Westergreen method.

3.4 Diff staining

Diff Staining in order to evaluate lymphocytes, monocytes, eosiophils and neutrophils was performed using Wright's staining.

3.5 Immunological testing

Both gel tubes containing culture environment with or without mitogen immediately were moved into incubator 37^{oC} and were kept for 16-24 hours. After this period of time tubes were removed from incubator and centrifuged at 2500 rpm for 15 minutes. Supernatants of this culture were collected and then concentration of cytokines including IL-4 and IFN-γ were measured by a sensitive sandwich ELISA kit (R&D system).

3.6 Statistical analysis

Statistical analysis was performed using SPSS software. Values of biochemical, hematological parameters and sedimentation rate in cupping and venous blood samples of each volunteer were analyzed using parametric T-Test measurement and immunological and diff factors were analyzed using non-parametric Wilcoxon measurement. Data were prepared as mean ± S.D and P value <0.05 was considered statistically significant and considered to be highly significant.

4. Results

4.1 Biochemical parameters (lipid profile, renal parameters, liver enzymes and other factors)

As shown in table 1, blood sample from cupping showed highly significant increase ($p<0.001$) in cholesterol, HDL, LDL and Triglyceride levels as compared to venous blood samples.

Table 1 also reveals highly significant increase ($p<0.001$) in serum uric acid level as compared to venous blood sample.

Blood sample from cupping showed insignificant increase ($p<0.001$) in serum SGOT and decrease ($p<0.001$) in serum SGPT levels.

Blood samples from cupping showed no insignificant difference ($p=0.121$) in serum CRP level in comparison to venous blood samples, while there was significant increase ($p=0.022$) in blood ferrous level as compared to venous blood samples.

4.2 Diff parameters and sedimentation factor

Table 2 reveals the comparison of monocytes, lymphocyte, neutrophills and eosinophills count between venous blood samples with samples obtained through the technique of cupping.

Blood sample from cupping showed highly significant decline in eosinophills ($p=0.032$), monocytes and neutrophills ($p=0.002$) and ESR ($p=0.013$) as well as significant increase ($p<0.001$) in lymphocytes as compared to venous blood samples.

4.3 Hematological parameters

Table 3 reveals the comparison of concentration of hematological parameters in blood samples.

Blood samples from cupping showed no significant statistical difference in WBC (p=0.151), MCH (p=0.076) and MCV (p=0.074), beside highly significant increase in RBC (p=0.002), hemoglobin (p=0.001), hematocrit (p=0.003), Mean Cell Hemoglobin Concentration (p=0.001) and viscosity (p=0.003) as well as significant decline (p<0.001) in platelet count as compared to venous blood samples.

4.4 Immunological parameters

The results of comparison of immunological factors (Table 4) showed that stimulatory index (which indicates difference between response after stimulation with PHA mitogen and basic response) in blood sample from cupping in order to production of IFN-γ (p=0.002) and IL-4 (p=0.001) were significantly less as compared to venous blood samples.

Factor	*From*	*Number*	*Mean*	*SD*	*p-value*
Uric Acid (ml %)	*Vein*	53	5.16	1.15	P<0.001
	Cupping		6.37	1.7	
Cholesterol (mg/dl)	*Vein*	53	142.39	32.83	P<0.001
	Cupping		171.39	35.59	
Triglyceride (mg/dl)	*Vein*	53	135.62	94.44	P<0.001
	Cupping		166.68	94.33	
LDL (mg/dl)	*Vein*	53	73.45	21.42	P<0.001
	Cupping		85.81	29.47	
HDL (mg/dl)	*Vein*	53	34.58	10.17	P<0.001
	Cupping		39.28	10.72	
SGOT (U/L)	*Vein*	53	23.71	10.3	P<0.001
	Cupping		34.49	19.4	
SGPT (U/L)	*Vein*	41	217.93	88.77	P<0.001
	Cupping		154.8	92.03	
CRP (mg/dl)	*Vein*	53	1.55	1.05	P=0.121
	Cupping		1.77	1.6	
Ferrous (µg/dl)	*Vein*	49	100.65	38.64	P=0.022
	Cupping		158.48	173.88	

Table 1. Comparison of Biochemical parameters between blood samples obtained intravenously through standard procedure, with blood samples obtained through the technique of cupping

Factor	*From*	*Number*	*median*	*Mean*	*SD*	p-value
Monocytes (%)	*Vein*	51	1	1.33	1.36	P=0.002
	Cupping	38	1	0.71	0.86	
Lymphocytes (%)	*Vein*	51	33	34.7	10.04	P<0.001
	Cupping	38	41	40.92	10.03	
Neutrophils (%)	*Vein*	51	62	62.67	11.27	P=0.002
	Cupping	38	58	57.92	10.26	
Eosiophils (%)	*Vein*	51	1	1.1	1.7	P=0.032
	Cupping	38	0	0.46	0.69	
ESR (mm)	*Vein*	42	-	7.15	3.66	P=0.013
	Cupping	42	-	2.57	2.5	

Table 2. Comparison of Diff parameters and Sedimentation Rate between blood samples obtained intravenously through standard procedure, with blood samples obtained through the technique of cupping

Factor	*From*	*Number*	*Mean*	*SD*	*p-value*
WBC (µl)	*Vein*	39	6.12×10^3	1.54×10^3	P=0.151
	Cupping		5.74×10^3	2.01×10^3	
RBC (µl)	*Vein*	39	5.56×10^6	0.14×10^6	P=0.002
	Cupping		5.63×10^6	0.75×10^6	
Hb (g/dl)	*Vein*	39	15.67	1.2	P=0.001
	Cupping		18.51	2.4	
HCT (%)	*Vein*	39	44.93	2.48	P=0.003
	Cupping		46.84	5.65	
MCHC (%)	*Vein*	39	34.84	1.51	P=0.001
	Cupping		35.01	1.85	
MCH (pg)	*Vein*	39	29.2	2.22	P=0.076
	Cupping		29.11	2.29	
MCV (fl)	*Vein*	39	82.19	4.25	P=0.074
	Cupping		82.61	5.38	
PLT (µl)	*Vein*	39	117.73×10^3	76.06×10^3	P<0.001
	Cupping		203.87×10^3	50.23×10^3	
Viscosity (cpo)	*Vein*	39	3.185	0.09	P=0.003
	Cupping		3.265	0.21	

Table 3. Comparison of hematological parameters between blood samples obtained intravenously through standard procedure, with blood samples obtained through the technique of cupping

4.5 Comparison of venous blood samples before and after cupping

Results of comparing the difference between venous blood samples before cupping and 2 weeks after cupping as regards biochemical, hematological and immunological parameters has been gotten in tables 5 to 8.

Factor	*mitogen*	*From*	*Number*	*median*	*Mean*	*SD*	*p-value*
IFN-γ (pg/ml)	No	*Vein*	53	38.66	538.06	2296.2	P=0.004
		Cupping	51	40.55	339.8	1001.8	
IFN-γ (pg/ml)	Yes	*Vein*	54	5825	8410.97	6525.4	P<0.001
		Cupping	51	1824.5	4449.73	6517.2	
IFN-γ Stimulatory Index (pg/ml)	-	*Vein*	45	8581	8148.07	5285.8	P=0.002
		Cupping	49	1809.7	4293.5	6571.6	
IL-4 (pg/ml)	No	*Vein*	53	17.2	119.43	440.64	P<0.001
		Cupping	51	21.83	162.75	510.17	
IL-4 (pg/ml)	Yes	*Vein*	53	63.52	231.79	668.79	P=0.003
		Cupping	53	29.36	290.69	1172.3	
IL-4 Stimulatory Index (pg/ml)	-	*Vein*	51	33.03	123.45	382.41	P=0.001
		Cupping	49	5.04	144.24	712.38	

Table 4. Comparison of Immunological parameters between blood samples obtained intravenously through standard procedure, with blood samples obtained through the technique of cupping

Factor	*Time*	*Number*	*Mean*	*SD*	*p-value*
Uric Acid (ml %)	*Before*	50	5.16	1.15	P=0.024
	After		5.46	1.2	
Cholesterol (mg/dl)	*Before*	50	142.39	32.83	P=0.245
	After		148.55	36.52	
Triglyceride (mg/dl)	*Before*	50	135.62	94.44	P=0.063
	After		157.39	97.47	
LDL (mg/dl)	*Before*	50	73.45	21.42	P=0.405
	After		74.5	18.7	
HDL (mg/dl)	*Before*	50	34.58	10.17	P=0.567
	After		35.69	10.00	
SGOT (U/L)	*Before*	50	23.71	10.3	P=0.253
	After		24.75	11.85	
SGPT (U/L)	*Before*	50	217.93	88.77	P=0.024
	After		235.8	94.6	
CRP (mg/dl)	*Before*	50	1.55	1.05	P=0.041
	After		2.03	1.65	
Ferrous (µg/dl)	*Before*	49	100.65	38.64	P=0.684
	After		97.93	31.38	

Table 5. Comparison of Biochemical parameters in venous blood samples before and 2 weeks after cupping

Factor	*Time*	*Number*	*median*	*Mean*	*SD*	*p-value*
Monocytes (%)	*Before*	51	1	1.33	1.36	P=0.648
	After	49	1	1.14	1.3	
Lymphocytes (%)	*Before*	51	33	34.7	10.04	P=0.643
	After	49	34	33.86	9.15	
Neutrophils (%)	*Before*	51	62	62.67	11.27	P=0.509
	After	49	64	64.16	9.57	
Eosiophils (%)	*Before*	51	1	1.1	1.7	P=0.842
	After	49	1	0.89	1.06	
ESR (mm)	*Before*	46	-	3.43	7.15	P=0.82
	After		-	4.15	4.09	

Table 6. Comparison of Diff parameters and Sedimentation Rate in venous blood samples before and 2 weeks after cupping

Factor	*Time*	*Number*	*Mean*	*SD*	*p-value*
WBC (µl)	*Before*	48	6.12×10^3	1.54×10^3	P=0.581
	After		6.23×10^3	1.74×10^3	
RBC (µl)	*Before*	48	5.56×10^6	0.14×10^6	P=0.04
	After		5.15×10^6	0.37×10^6	
Hb (g/dl)	*Before*	48	15.67	1.2	P=0.045
	After		15.12	1.25	
HCT (%)	*Before*	48	44.93	2.48	P=0.026
	After		43.13	2.71	
MCHC (%)	*Before*	48	34.84	1.51	P=0.455
	After		35.02	1.27	
MCH (pg)	*Before*	48	29.2	2.22	P=0.709
	After		29.41	1.75	
MCV (fl)	*Before*	48	82.19	4.25	P=0.35
	After		84.02	3.94	
PLT (µl)	*Before*	48	117.73×10^3	76.06×10^3	P=0.613
	After		207.46×10^3	61.1×10^3	
Viscosity (cpo)	*Before*	48	3.185	0.09	P=0.026
	After		3.11	0.1	

Table 7. Comparison of Hematological parameters in venous blood samples before and 2 weeks after cupping

Factor	*mitogen*	*Time*	*Number*	*median*	*Mean*	*SD*	*p-value*
IFN-γ (pg/ml)	No	*Before*	53	38.66	538.06	2296.23	P=0.425
		After	48	38.2	524.27	2250.69	
IFN-γ (pg/ml)	Yes	*Before*	54	5825	8410.97	6525.48	P=0.18
		After	47	6495	7646.93	5922.43	
IFN-γ Stimulatory Index (pg/ml)	-	*Before*	45	8581	8148.07	5285.8	P=0.857
		After	48	6432.1	162.43	4762.86	
IL-4 (pg/ml)	No	*Before*	53	17.2	119.43	440.64	P=0.506
		After	47	20.3	241.47	482.5	
IL-4 (pg/ml)	Yes	*Before*	53	63.52	231.79	668.79	P=0.192
		After	47	69.81	7112.07	629.43	
IL-4 Stimulatory Index (pg/ml)	-	*Before*	51	33.03	123.45	382.41	P=0.253
		After	47	30.49	75.87	165.61	

Table 8. Comparison of Immunological parameters in venous blood samples before and 2 weeks after cupping

5. Discussion

Community surveys carried out over the past decade document that more than one-third of Americans use complementary and alternative medicinal treatments in a given year (D. M. Eisenberg, et al., 1993). There is reason to believe that the use of complementary and alternative therapies is more common among people with psychiatric problems than the rest of the population because fatigue, insomnia, chronic pain, anxiety, and depression are among the most commonly reported reasons for the use of complementary and alternative therapies in community surveys (D. M. Eisenberg, et al., 1998). In fact, it's estimated that up to 80 percent of the U.S. population has tried a therapy such as acupuncture or mind/body medicine, and nearly 40 percent of all Americans rely on some type of alternative therapy as part of their regular healthcare regimen (Fontanarosa & Lundberg, 1998; Kessler, et al., 2001). Despite the prominent role of modern medicine in diagnosis and management of many diseases, most of the times it is unable to control and prevent chronic disease. Many researchers have proven the efficacy of complementary and alternative medicine in this regard and such medical aspect is considered as integrative medicine (Hashem Dabbaghian, Gushehgir, & Siadati, 2008).

As mentioned before, cupping has worldwide been used in traditional medicine systems and is also nowadays used as complementary or alternative therapy especially in patients with pain syndromes. Recent clinical studies have reported efficacy in patients with brachialgia paresthetica nocturna, carpal tunnel syndrome, cancer pain and lower back pain (Farhadi, et al., 2009; Ludtke, Albrecht, Stange, & Uehleke, 2006; Michalsen, et al., 2009). Although many physicians use and counsel this skill of remedy, but there are lots of incomprehensible aspects about mechanism of cupping action. Greeting of many people to use this kind of remedy (Hashem Dabbaghian, et al., 2008) have led to the need for further

investigations about confirmation or restitution of cupping impressiveness, as well as its comparison with modern medicine. Since some researchers believe there is no difference between venous blood samples and deriving blood of cupping in the respect of hematological and biochemical factors, it become necessary to compare components of cupping and venous blood.

5.1 Biochemical factors

Cholesterol is a chemical compound that is naturally produced by the body and is a combination of lipid (fat) and steroid. Cholesterol is a building block for cell membranes and for hormones like estrogen and testosterone. About 80% of the body's cholesterol is produced by the liver, while the rest comes from our diet. The liver is able to regulate cholesterol levels in the blood stream and can secrete cholesterol if it is needed by the body. LDL cholesterol is called "bad" cholesterol, because elevated levels of LDL cholesterol are associated with an increased risk of coronary heart disease. LDL lipoprotein deposits cholesterol on the artery walls, causing the formation of a hard, thick substance called cholesterol plaque. Over time, cholesterol plaque causes thickening of the artery walls and narrowing of the arteries, a process called atherosclerosis. HDL cholesterol is called the "good cholesterol" because HDL cholesterol particles prevent atherosclerosis by extracting cholesterol from the artery walls and disposing of them through the liver. Thus, high levels of LDL cholesterol and low levels of HDL cholesterol (high LDL/HDL ratios) are risk factors for atherosclerosis, while low levels of LDL cholesterol and high level of HDL cholesterol (low LDL/HDL ratios) are desirable (Hall. & Edward, 2011). Niasri and colleagues in order to determine if a reduction in serum lipoproteins, especially LDL cholesterol, is a preventive approach against atherosclerosis; investigated the effects of wet cupping on serum lipoprotein concentrations (since phlebotomy has been a recommended method to reduce serum lipoprotein levels). In this randomized controlled trial, men in the treated group were subjected to wet cupping, whereas men in the control group remained untreated. The serum concentrations of lipids, collected from brachial veins, were determined at the time of wet cupping and then once a week for 3 weeks. A substantial decrease in LDL cholesterol and in the LDL/HDL ratio was found in the treated group compared to the control. There were no significant changes in serum triglyceride between groups. Although there were no statistically significant variations in total cholesterol and HDL cholesterol, a 7% decrease in total cholesterol and 3% increase in HDL cholesterol may be clinically important. And they finally concluded that wet cupping may be an effective method of reducing LDL cholesterol in men and consequently may have a preventive effect against atherosclerosis (Niasari, et al., 2007). In this regard, our results showed concentrations of HDL, LDL, TG and cholesterol in cupping blood were significantly higher as compared to venous blood. So it can be suggested that cupping plays an important role in decreasing risk factors of atherosclerosis by further extrusion of lipids. It is well understood that bloodletting (especially when it is prolonged and repeated) is associated with a reduction in cardiovascular events (Meyers, Jensen, & Menitove, 2002). Such results have been found in other studies which can confirm this hypothesis (Niasari, et al., 2007). Bloodletting has been a recommended method to reduce serum lipoprotein concentrations. In the study of Gugun and colleagues all of subjects were treated with cupping in one time and a significant LDL cholesterol increase ($P < 0.0000$) was found in almost subjects, moreover there was strong positive correlation between LDL cholesterol pre and one hour

post cupping (r= 0.987); finally they concluded that cupping will increase the level of LDL cholesterol an hour after treatment (Gugun & Alfian., 2010). In present study by reduplication of blood sampling from vein 2 weeks after cupping, we did not find any change in lipoproteins concentrations in venous blood. This result was different from another study which showed reduction of LDL and cholesterol beside HDL increase and no change of triglyceride (Niasari, et al., 2007). So it seems more probably that prolonged duration of the study and repetition of cupping are the reason for such difference and this needs further considerations.

An initial step in detecting liver damage is a simple blood test to determine the presence of certain liver enzymes (proteins) in the blood. Under normal circumstances, these enzymes reside within the cells of the liver. But when the liver is injured for any reason, these enzymes are spilled into the blood stream. Among the most sensitive and widely used liver enzymes are the aminotransferases. They include aspartate aminotransferase (AST or SGOT) and alanine aminotransferase (ALT or SGPT). These enzymes are normally contained within liver cells. If the liver is injured or damaged, the liver cells spill these enzymes into the blood, raising the enzyme levels in the blood and signaling the liver disease. AST (SGOT) is normally found in a diversity of tissues including liver, heart, muscle, kidney, and brain. It is released into serum when any one of these tissues is damaged. For example, its level in serum rises with heart attacks and with muscle disorders. It is therefore, not a highly specific indicator of liver injury; ALT (SGPT) is, by contrast, normally found largely in the liver. This is not to say that it is exclusively located in liver, but that is where it is most concentrated. It is released into the bloodstream as the result of liver injury. It therefore serves as a fairly specific indicator of liver status (Wong, Ooi, & Ang, 2000). Comparison of venous blood with cupping blood as regards of SGOT concentration showed its level in cupping blood was as well above venous blood. It should be noted that this increase was mild and is not probably as a result of liver injury; however it seems this increase is due to this opinion that blood of cupping is tugged from interstitial tissue fluid and capillaries in which enzyme levels differ from whole blood of venous. Beside, hemolysis happens inevitably after cupping; therefore we can expect false increase of SGOT by cupping (Desai, 2004; Fallah, 2007; Friedman & Young, 1997). Nevertheless, SGPT level was reduced in cupping samples as compared to venous samples, and such result is concomitant with Bilal and his colleagues findings (Bilal, et al., 2011). Moreover, reduplication of blood sampling from vein 2 weeks after cupping in present study did not cause any change in SGOT level in venous blood but there was significant increase of SGPT in plasma after 2 weeks. The effect of cupping on improving blood and lymph circulation and strengthening organic (liver) function was introduced in the study of Jun-Ru (Jun-ru, Cai-xia, & Tian-ran, 2007). Such finding is not in accordance with the point that liver damage causes SGPT increase (Wong, et al., 2000); i.e. it seems the augmentation of SGPT after cupping is not as a result of liver impairment and more probably other factors like skin injury after cupping are related to this increase.

CRP is used mainly as a marker of inflammation. Apart from liver failure, there are few known factors that interfere with CRP production (Pepys & Hirschfield, 2003). The erythrocyte sedimentation rate (ESR) is a simple and inexpensive laboratory test that is widespread in clinical practice for assessing the inflammatory or acute response (Plebani &

Piva, 2002). The ESR can be used in the diagnosis of inflammatory conditions, as well as in the prognosis of non-inflammatory conditions (Saadeh, 1998). Generally, ESR does not change as rapidly as CRP, either at the start of inflammation or as it goes away. Since CRP is not affected by as many other factors as is ESR, making it a better marker of inflammation (Ismailov, Shevchuk, & Khusanov, 2005) and measuring CRP values can be useful in determining disease progress or effectiveness of treatments. Our results showed there was no difference between cupping blood samples and venous blood samples from the view point of CRP level, however cupping sedimentation rate was significantly less than venous ESR; it seems such result is in accordance with the concept that ESR is affected by various factors. So ESR decline can be considered as a result of high density of cupping blood samples in which RBC's number is much more than venous blood samples or total proteins of cupping blood have been decreased as compared to venous blood (Ismailov, et al., 2005). Beside, reduplication of cupping after 2 weeks has caused CRP level increase which is against Ahmed and colleagues findings (Ahmed, et al., 2005); however 2 weeks after cupping there was no difference regarding to venous ESR. As mentioned before, CRP changes are as specific as ESR changes (Ismailov, et al., 2005) and it seems tissue injury by performing of cupping contributes in increase of CRP levels after 2 weeks.

In our study, uric acid level in cupping blood samples was greater than venous blood as same as findings about Serum iron level. Iron (Fe) activates xanthine oxidase (XO), eventually the high Fe results in more active XO and higher uric acid levels. It has proposed cupping have more efficacies in excretion of superfluous elements as compared to blood sampling from venous, as excess Fe can be eliminated through phlebotomy (blood donation) and reducing urate levels (Brand, McGee, Kannel, Stokes, & Castelli, 1985; Iblher & Stark, 2007; Khosla, et al., 2005; Miller, Grover, Nayini, & Aust, 1993). Cupping has been implied in high risk patients for heart disease in order to discharge excess iron of the body because iron contributes to cardiovascular and coronary heart disease and that iron decline may protect against heart attacks (Dastjerdi, 1996; Prabhu, Prabhu, & Prabhu, 2009). Evaluation of venous blood samples 2 weeks after cupping showed no change of iron despite there was uric acid increase next to this time.

5.2 Hematological factors

It has been reported WBCs count in cupping blood samples is one tenth of their count in venous blood samples (Sheykhu, 2008). Our results also showed WBCs count decline despite this decrease was not statistically significant. In addition, there was no increase in WBCs count after 2 weeks and it seems if we repeated cupping for other times, WBCs count would increase similar other studies (Ahmed, et al., 2005).

RBCs count and Hb concentration in cupping samples were significantly well above of those in venous blood samples and this reveals cupping blood is so dense. Since these RBCs morphology differs from venous RBCs, cupping more probably plays an important role in excretion of old RBCs (Sheykhu, 2008). The relationship is that when red blood cells increases, hematocrit increases, and blood viscosity also increases because too much red blood cells (erythrocytes) in the blood makes the blood more dense/thicker, and therefore slows down the flow of the blood. In short, blood cells, hematocrit, and blood viscosity are

all directly proportional with each other (Galduroz, Antunes, & Santos, 2007). Our results can be as a confirmation to this proportion: HCT (Hematocrit) percent and viscosity in cupping blood samples were also well above venous blood samples. It seems plausible that in the hematological system, cupping can regulate coagulation and anti-coagulation systems (e.g., decrease the level of hematological element such as fibrinogen) as well as decrease the HCT (Ahmadi, Schwebel, & Rezaei, 2008). In this survey, platelets counts in cupping blood as compared to venous blood were also above; it seems sucking pressure due to cupping have caused further discharge of low density platelets, this platelets excretion increase blood's clotting time, by these the flow of blood and the end organ oxygenation will be increased (Ahmadi, et al., 2008). Additionally, 2 weeks after cupping, HCT and Hb level and viscosity as well as RBCs count in venous blood was decreased significantly; such results means decrease of viscosity following RBCs count decline, which causes reduction of cardiac load (S. Eisenberg, Horn, & Nelson, 1964).

RBCs transport hemoglobin which, in turn, transports oxygen. The amount of oxygen tissues receive depends on the amount and function of RBCs and hemoglobin. The MCV reflects the size of red blood cells. The MCH and MCHC reflect the hemoglobin content of red blood cells. The values for MCHC, and MCH are calculated from the hemoglobin (Hgb), hematocrit (Hct), and RBC count (Zuckerman, 2007):

$$MCHC = Hgb/Hct$$

$$MCH = Hgb/RBC\ count$$

In our study there was no statistical difference as regards of MCV and MCH, however MCHC levels were more above in cupping blood than venous blood, it means in spite of equality in hemoglobin percent, hemoglobin concentration in RBCs of cupping blood samples was highest.

5.3 Immunological factors

It has been proposed that cupping can likely affect the immune system via 3 pathways: (a) Irritation of the immune system by making an artificial local inflammation, and then activate the complementary system and increase the level of immune products such as interferon and TNF (Tumor Necrotizing Factor); (b) effect the thymus; and (c) control traffic of lymph and increase the flow of lymph in lymph vessels (Ahmadi, et al., 2008).

As mentioned before, for comparing TH1/TH2 response in blood samples of venous and cupping we evaluated IFN-γ and IL-4 concentrations in supernatant of vein and cupping blood cultures with or without the presence of HPA mitogen. Our results showed IFN-γ and IL-4 concentrations in cupping blood samples as compared to venous blood samples without presentation of any mitogen were well above; it seems the high level of lymphocytes in cupping blood samples plays an important role in discharge of IFN-γ and IL-4. However the main result of our study was that in the presence of HPA mitogen, concentration of IFN-γ and IL-4 in cupping blood samples were as less as venous blood samples; and we presumed that lymphocytes in cupping blood samples may do not have their natural function, so they cannot properly respond to stimulation of mitogen. Moreover, 2 weeks after cupping we did not see any difference in IFN-γ and IL-4

concentrations in venous blood; it seems by reduplication of cupping immune response will be affected and IFN-γ and IL-4 concentrations will increase (Ahmed, et al., 2005).

6. Conclusion

By assessing the uniform pattern of tested parameters of cupping blood samples in comparison to venous samples; it can be assumed that there is a marked difference in the composition of blood drawn through cupping as compared to the blood drawn intravenously. Such difference can be referred to the technique or the site of cupping; as in traditional medicine books it has been mentioned about therapeutic effects of cupping in different sites of the body (Ibn-e-Sina, 1997; Jorjani, 1975). Moreover cupping can summon immune cells and create inflammation at the site of hijama and helps to increase of blood circulation to different areas of the body which need medication (Ullah, Younis, & Wali, 2007). So according such evidence we can allege that hijama is not just a simple technique of taking blood, but it is as a curative procedure regarding traditional medical and Islamic books (Ibn-e-Sina, 1997; Jorjani, 1975).

Since the concentration of discharged biochemical and immunological factors in cupping blood is so high, this collected blood is not useful for management of disease.

The reason for the significant difference between cupping and venous blood samples is yet not known, however it can be assumed that it may be due to the presence of some unidentified substance in the blood samples from cupping site; so we cannot comment about advantages and disadvantages of it and we just can confess that these are the first steps to search about cupping mechanism and further investigations seems more crucial.

7. Suggestions

In this regard we suggest that:

1. As our research was just about clinical and laboratory changes of blood elements after cupping and there was no investigation about volunteers' satisfaction, so we offer planning of questionnaires for evaluating individuals' approbation about cupping efficacy.
2. This research was conducted just in healthy people with no previous disease, so we recommend searching about cupping effects on patients with specific diseases.
3. Our results are presented after one time cupping with a duration of 2 weeks, we offer planning of a proposal about doing cupping in different intervals to have a good investigation of cupping Time-Response.

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