Original Article *Spatula DD. 2013; 3(2):51-57*

Investigation of Selected Immunocytogenetic Effects of Wet Cupping in Healthy Men

Sağlıklı Erkeklerde Islak Kupa Çekiminin Seçilmiş İmmunositogenetik Etkilerinin Araştırılması

Ahmad Mohammad Khalil[1*], *Khaled Mahmoud Al-Qaoud*[2] *and Hiba Mohammad Shaqqour*[2]

[1]Department of Biology, Faculty of Science, Taibah University, Almadina Almunawwarah, Kingdom of Saudi Arabia. [2]Department of Biological Sciences, Yarmouk University, Irbid-Jordan.

SUMMARY

AIM: This present study was carried out to evaluate effect of cupping therapy using selected immunocytogenetic parameters.

METHODS: Forty four males participated as two groups; group I (age 21 ± 1 year) of 30 persons (23 experimental, 7 control) and group II (age over 40 years) of 14 subjects (9 experimental and 5 control). The experimental group received cupping treatment while the control group did not. Basic wet cupping was performed by a practitioner. Peripheral blood samples were collected over a month period; one day before cupping, one week after cupping and a third one month follow up sample was obtained to determine longer term carryover of the possible effects of cupping. White and red blood cells (WBC and RBC) count and hemoglobin (Hb) concentration were measured. 50% Hemolytic Complement (CH50) activity was conducted. Chromosomes were prepared using a colchicines-fixative-air drying-Giemsa schedule. The Cell Replication Index (CRI) and Sister Chromatid Exchanges (SCE) frequencies were recorded.

RESULTS: Levels of SCE did not significantly differ between experimental and control groups. The same applied to CRI. Although WBC counts were significantly higher in persons after wet cupping, this was not the case for the number of RBCs and Hb concentration. Complement activity was enhanced by cupping in the older group but not in the younger one.

CONCLUSIONS: No correlation between cupping and cytogenetic parameters (CRI and SCE) was observed. However, cupping seems to play a role in activation of complement system as well as modulation of cellular part of immune system.

Key words: Complement activity; Complementary Medicine; Cupping therapy; Sister chromatid exchanges.

ÖZET

AMAÇ: Bu çalışma, selektif immünositogenetik parametreler kullanarak, kupa tedavisinin etkilerini değerlendirmek için gerçekleştirildi.

YÖNTEM: İki grup olarak 44 erkek çalışmaya katıldı. Grup 1 (21±1 yaş), 30 kişiden (23 deney, 7 kontrol); grup 2 (40 yaş üstü) ise 14 kişiden (9 deney, 5 kontrol) oluşturuldu. Deney grubundakilere kupa tedavisi uygulanırken, kontrol grubuna uygulanmadı. Temel ıslak kupa çekimi bir pratisyen tarafından gerçekleştirildi. Periferal kan örnekleri bir aylık süre boyunca biriktirildi. Kupa tedavisinin muhtemel etkilerinin uzun dönem sonuçlarını saptamak için, kupa çekiminden bir gün once, bir hafta sonra ve ayda üç kez tekrarlanmak üzere numuneler toplandı. Beyaz ve kırmızı kan hücreleri sayımı (WBC ve RBC) ve hemoglobin (Hb) konsantrasyonu ölçüldü. %50 hemolitik kompleman (CH50) aktivitesine bakıldı. Kromozomlar, bir kolşisin-fiksatif-hava kurutma-Gimsa süreci ile hazırlandı. Hücre replikasyon indeksi (CRI) ve kardeş kromatid değişimi (SCE) sıklıkları kayıt edildi.

BULGULAR: SCE seviyeleri bakımından deney ve kontrol grupları arasında anlamlı fark yoktu. Aynı durum CRI içinde geçerliydi. WBC sayısı, ıslak kupa çekimi sonrası anlamlı olarak yüksek olmasına rağmen, RBC ve Hb sayısı için aynı durum söz konusu değildi. Kompleman aktivitesi genç gruptan ziyade yaşlı grupta ıslak kupa çekimi ile kuvvetlendirildi.

SONUÇ: Kupa çekimi tedavisi ve sitogenetik parametreler (CRI ve SCE) arasında korelasyon gözlenmedi. Ancak, kupa çekiminin, immun sistemin hücresel parçasının düzenlemesi yanında kompleman sisteminin aktivasyonunda bir rol oynadığını göstermektedir.

Anahtar kelimeler: Kompleman aktivitesi, tamamlayıcı tıp, kupa tedavisi, kardeş kromatid değişimi.

Corresponding Author:
Prof. Ahmad M. Khalil,
Department of Biological Sciences, Yarmouk University,
Irbid-Jordan.
E-mail: kahmad76@yahoo.com.

Received May 30, 2013; accepted July 12, 2013
DOI 10.5455/spatula.20130712050838
Published online in ScopeMed (www.scopemed.org).
Spatula DD. 2013; 3(2):51-57.

INTRODUCTION

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. 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 [1, 2]. 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). In the UK, the practice of cuping therapy (CT) also dates back a long way with one of the leading medical journals 'The Lancet' being named after this practice [1, 2]. Now, cupping is available from acupuncturists and some chiropractors and massage therapists in the U.S. CT is divided into two broad categories: dry cupping and wet cupping (WC). The dry cupping (suction only) is the basic suction method of CT. In the second type, the skin is incised before the suction of the cups is applied [1, 3-6].

Cupping therapy is a form of Complementary and Alternative Medicine that has been successfully utilized for treatment of several conditions [5, 7-12]. Each kind of CT may be used for different purposes of treatment. Interest in cupping has increased recently and this issue substantially stimulated further cupping research. Lauche and his co-authors [9] published a 50-person German study and found that a single WC treatment on average significantly reduced chronic neck pain three days after the treatment, compared with a control group that had no treatment. Cao et al. [11] presented 550 studies published between 1959 and 2008. Wet cupping was used in majority of them. Compared to medications alone, CT combined with acupuncture plus medications was significantly better on pain relieving, but no difference in symptom improvement was reported. They emphasized the necessity for improvement of the methodological quality and standardization of the study design.

CT has no serious side effects aside from minimal discomfort [2, 11] due to the method of application of skin cuts to the patient.

WC was designed to clean the body of impurities and excess fluid. Like blood donation [2] which is believed to reduce the amount of toxic substances (mercury, pesticides, fire retardants…), it may elicit the release of morphine like substances (endorphins), serotonin or cortisol. This can ultimately lead to pain relief and alter the physiological status of the individual [13]. It is therefore very logical to speculate that removal of such materials from the body will improve immune response and reduce the frequency of sister chromatid exchanges (SCE). The latter is a sensitive index of DNA damage resulting from exposure to endogenous and exogenous toxins [14]. The first part of the present study was designed for assessment of the possible effects of WC on selected components of the immune system; Complete Blood Count (CBC), complement activity by investigation of the 50 % Hemolytic Complement (CH50) and Mixed Lymphocyte Reaction (MLR) assay. The complement system plays a major role in host defense against infection and inflammatory processes. It consists of more than 25 self-assembling proteins as well as a series of receptors and regulatory proteins. CH50 assay evaluates the function of the C1 through C9 complement proteins [15].

The second part aimed at the possible correlation between WC and two cytogenetic parameters; SCE and Cell Replication Index (CRI). Because WC is favored in the Middle East and Eastern Europe [2], it was applied in this research. Based on the fact that cupping massage can be easily used at home and on the expectation that it may become a practice to everyday life to improve the general physiological status, healthy subjects were used in this investigation. As we age, especially after 40 years old, our body begins to break down. This includes loss of muscle, adhesions in fascia and decrease in blood flow, so a second (older) group was included. No research has yet been conducted into the effects of a home-based cupping massage program in relation to prevention and health care.

MATERIAL AND METHODS

Subjects

The study was performed on 44 healthy men from Irbid City (North of Jordan). Subjects were recruited utilizing a range of advertising techniques including university email system. The participants were randomly allocated to respective groups (either treatment or control group). The subjects were informed about the details of the research plan and consent was signed after completing a specially designed questionnaire and before commencing the study. Ethical approval was sought from Yarmouk University Research Committee.

Inclusion Criteria

- Subjects over 20 years old with no health problems.
- Subjects with no smoking history.
- Subjects with no back or neck pain.
- Subjects neither exposed to diagnostic X-ray nor treated with drugs over the 3 months preceding the experiment.

Exclusion Criteria

- Subjects suffering from serious heart troubles.
- Pregnant women.
- Cancer patients.
- Subjects with muscle spasms at the cupping region.

Cupping Procedure

A hygienic WC procedure was carried out between 12:00 noon and 2:00 pm by a specialized practitioner who was regularly performing cupping in clinical settings utilizing a hand suction pump, plastic cups of the same size and anti-septic tools according to the protocol described by Niasari et al. [16]. In all cases, the cups were applied at the trapezius muscle between the two scapula regions (Figure1). This muscle spans the neck, shoulders and upper back, the area where muscle tension and myogeloses most commonly occur. Negative suctioning was applied before skin puncturing (5 incisions, 1 mm depth and 2-4 mm length) by a razor blade. The cup was replaced on the same site for 20 min until it was filled with blood. The volume of sucked blood was about 20-25 ml for each donor. The skin was cleaned and left to dry.

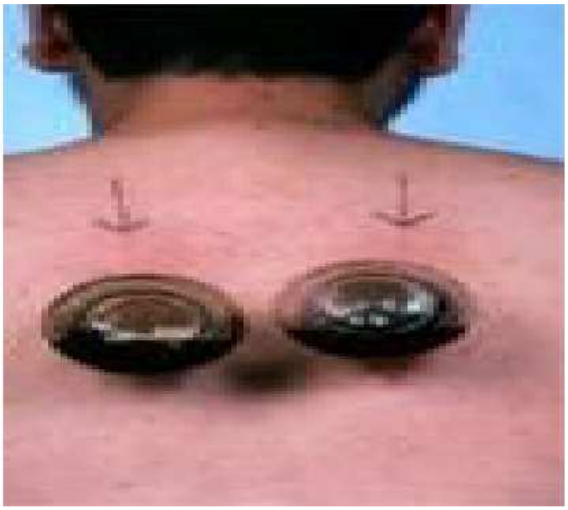

Figure 1. Application of wet cupping

Blood Collection

From each volunteer, 7 ml of venous blood were collected in heparinized tubes, while 2 ml were contained in a plain plastic tube for serum preparation according to the following schedule: one day before cupping, one week after cupping and one month after cupping. The person did not receive any medication or exposed to X-ray during the whole cupping period. Three blood samples were obtained from controls as the treated groups. Immediately, 200 µl of whole blood from each donor were used for determination of CBC and Hemoglobin (Hb) concentration by using 18 parameters automated hematology analyzer machine (Mythic, Orphee S.A). Serum samples were frozen at -70 ºC to do analysis in one setting.

CH50 Assay

Sheep red blood cells coated with antisheep erythrocyte antibody were used. A series of two fold serial dilutions of control and test serum in buffered saline each in duplicate were prepared and after 80 min incubation at 37 ºC centrifuged. Concentration of Hb released from destroyed Red Blood Cells (RBC) was measured in the supernatant at 540 nm.

Mononuclear Cells (MNC) Preparation and MLR Assay

2.5×10^6 cells/ml of responder MNC were cultured in 96 well culture plates with 3.33×10^6 cells/ml of stimulator cells (splenocytes) or with media alone. The cells were incubated in a humidified 37°C, 5% CO_2 incubator for 120 h. The cells were harvested and the responder cell reactivity was measured by the classical MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) proliferation assay. The absorbance was measured at 540 nm on a microplate reader, with background reading at 690 nm.

Cell Culture and Chromosome Preparation

This was done as described previously [14]. Briefly, about 2.0×10^6 cells / ml were placed in a final volume of 10 ml culture medium in 25 cm^2 flasks. The medium consisted of RPMI 1640 buffered with 25 mM HEPES and supplemented with 15% fetal calf serum, 50 IU penicillin, 50 µg/ml streptomycin and 0.5 % phytohemeagglutinin-M. Bromodeoxyuridine (ACROS Organics; USA, final concentration 30 µg/ml) was added at the initiation time. To rigidly control the technical aspects of the experimental protocol throughout the study and to ensure the responsiveness of the test system, a well known SCE-inducing agent; mitomycin C (MMC, 20

ng/ml) was included as positive. Four cultures were made for each treatment. Cultures were incubated at 37 °C for 72 h. Colcemid (10 µg/ml) was added in the last 2 h to stop mitotic at metaphase. Cells were harvested, swollen in a hypotonic solution (0.056% KCl) for 20 min, and fixed in Carnoy solution (absolute methanol: glacial acetic acid; 3:1). The cells were spread out onto a chilled slide. To visualize SCE (Figure 2), Fluorescence-plus-Giemsa (FPG) technique was used. The slides were treated with Hoechst stain, then exposed to UV light for 35 min and stained in Giemsa solution.

The mean SCE/cell was recorded on the basis of 20-30 clearly differentiated second metaphase (M2) cells containing 44±2 chromosome/spread for each sample. The CRI was calculated by finding the percentage of the first (M1), second (M2) and third (M3) metaphases among 200 dividing cells as follows:

CRI= [%M1+2(M2%) +3(%M3)]/100.

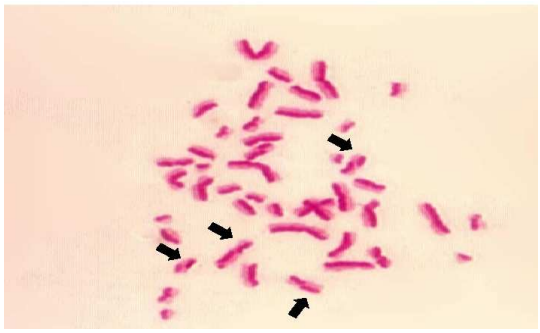

Figure 2. A chromosomal spread representing blood lymphocytes from persons subjected to wet cupping. 46 chromosomes are seen, four of them with SCE. Arrows show sites of SCE.

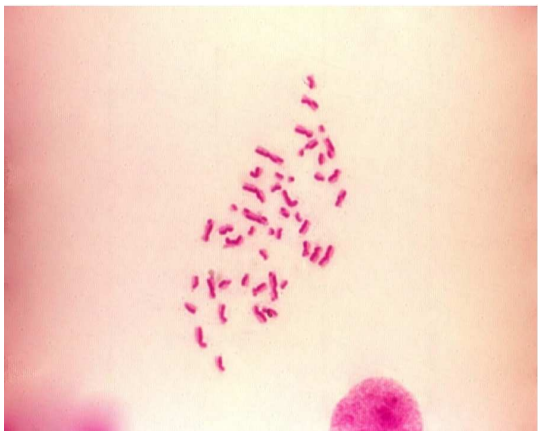

Figure 3. A chromosomal spread of a lymphocyte from a culture treated with the positive mitomycin C (MMC) showing 44 chromosomes and 14 SCE.

Data Analysis

The paired sample t-test was employed to determine the difference between data collected from subjects before and after cupping. The level of significance was set at 5 %. All data analysis was performed using Statistical Package for Social Sciences (SPSS) v.21 for windows.

RESULTS

In this study, participants were asked to report any side effects during the treatment period, no major side effects were recorded aside from minimal discomfort and the feeling of slight headedness similar to the sensation one feels after having had blood taken from him. None of the participants in the treatment group discontinued treatment because of worsening symptoms.

The frequency of SCE among individuals performed WC regardless of time of blood collection; one day before cupping, one week or one month after cupping, ranged from 4.52±1.8 SCE/cell. This is not statistically significant ($P>0.05$) compared to the level recorded in lymphocytes from the blood taken one day before cupping. In contrast, the positive control (20 ng/ml MMC) induced highly significant rate of SCE; 19.90±5.6. The results are summarized in table (1) as well as in figures 2 and 3.

A similar trend is noticed in the values of CRI; where the indices varied between 2.08±0.3 and 2.23±0.2 in the experimental group. These values are not significant ($P>0.05$) compared to that before cupping and to the positive control mean value as well (Table 1).

In the older group (>40 years), slight, but not significant, elevations in SCE and reductions in CRI were observed (data not shown) as compared to the corresponding readings recorded in the younger group.

Inconsistent and non significant ($P>0.05$) differences in the number of RBC and Hb concentration, between the experimental groups and their corresponding controls were observed. However, the total White Blood cells (WBC) counts were significantly ($P<0.05$) in the population after receiving cupping treatment as compared to the control and the experimental subjects before cupping (Figure 4). Furthermore, in both age groups, the number of granulocytes was significantly elevated after one month of cupping application relative to controls and experimental either before cupping or one week after cupping (Figure 5). In both age groups, cupping resulted in significant ($P<0.05$).

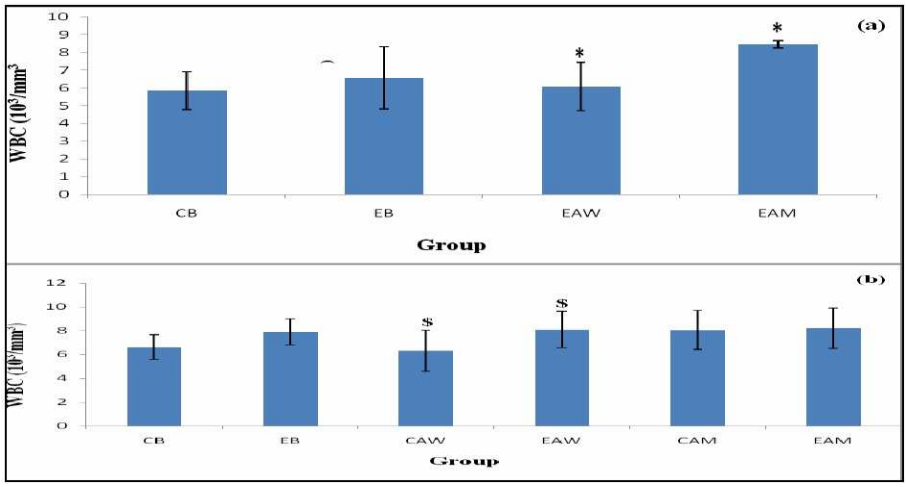

Figure 4. The effect of wet cupping on total White Blood Cell (WBC) count. (a) Age group I (23 ± 1 year). (b) Age group II (> 40 years). Control (C); before one day (CB); after one week (CAW); after one month (CAM); Experimental group (E); before one day (EB); after one week (EAW); after one month (EAM). *, $: Significant at $P < 0.05$ level.

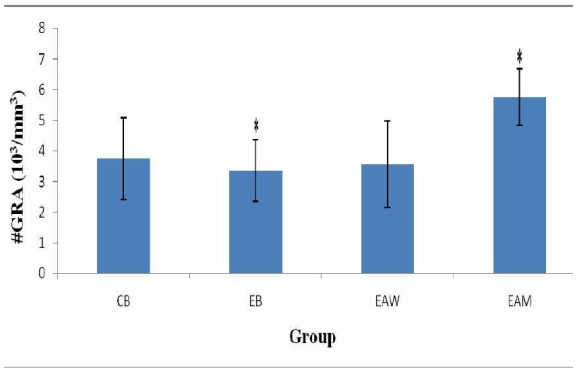

Figure 5. The effect of wet cupping on granulocytes (GRA) counts in both age groups. Abbreviations are as in legend of Figure 4.

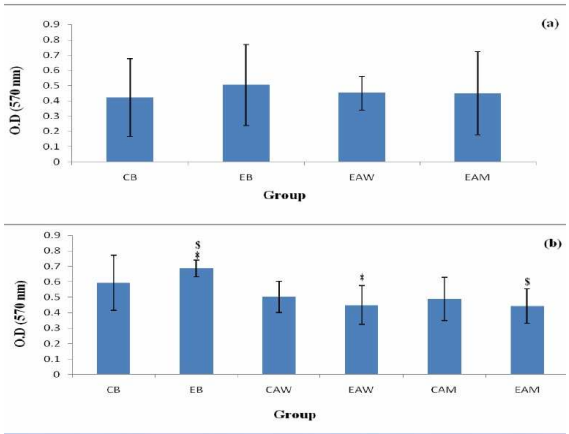

Figure 6. Optical Density (O.D) for cell proliferation of peripheral blood lymphocytes against xenogenic cells. (a) Age group I (23±1 year). (b) Age group II (> 40 years). Abbreviations are as in legend of Figure 4.

Table 1. The rate of sister chromatid exchanges (SCE) and replication index (CRI) in blood lymphocytes from young healthy men (age 21±1 year) before and after wet cupping. Values represent means of readings from four cultures.

Treatment	CRI (mean ± SD)	SCE (mean ± SD)
Control group (C)		
Before one day (CB)	2.25 ± 0.1	3.88 ± 0.8
After one week (CAW)	ND	ND
After one month (CAM)	ND	ND
Experimental group (E)		
Before one day (EB)	2.12 ± 0.3	4.64 ± 1.1
After one week (EAW)	2.23 ± 0.2	4.52 ± 1.8
After one month (EAM)	2.08 ± 0.3	4.62 ± 1.6
Mitomycin C (Positive Control; 20 ng/ml)	1.90 ± 0.3*	19.90 ± 5.6*

ND: Not determined.
* Statistically significant ($P < 0.05$).

reductions in lymphocytes counts in comparison with the controls and experimental one day before cupping (Figure 6). Finally, although clear enhancement (Figure 7 a) of the complement activity was not observed in young subjects undergoing cupping, this was obvious in the older group (Figure 7 b).

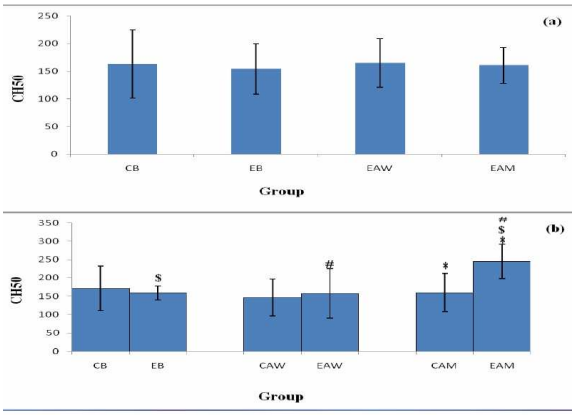

Figure 7. The effect of wet cupping on 50% Hemolytic Complement (CH50) activity. (a) Age group I (23 ± 1 year). (b) Age group II (> 40 years). Abbreviations are as in legend of Figure 4.

DISCUSSION

One finding of this randomized controlled trial, where men in the treated group were subjected to WC, whereas men in the control group remained untreated, is that there is no correlation between wet cupping and cytogenetic parameters (CRI and SCE). To our knowledge, this is the first study trying to evaluate the cytogenetic effects of WC and the results may represent the first steps to searching the genetic aspects of CT. The present results may suggest that the metabolites drawn and discarded through cupping, and removal of which might be creating a favorable balance between various vital parameters [17], are not mutagenic using SCE bioassay. Alternatively, WC may be protective against SCE formation by activating antioxidant defence mechanisms which remove oxidant damage. However, the latter interpretation is speculative and the hypothesis needs further elucidation. Other explanations, for example unspecific treatment relieving effects such as changes of the hormonal and the emotional status could not yet be ruled out. Future research, both in healthy people with no previous disease and in patients with specific diseases, should examine other cytogenetic endpoints such as micronucleus test or chromosome aberrations assay. Such studies can be conducted on venous blood samples and on blood samples from cupping site.

The other main outcome of this study is that wet cupping seems to play a vital role on activation of complement system. Wet cupping is also effective on some of the cellular part of the immune system. In general, cupping increased counts of WBC. Similar rises in the WBC counts were reported before [18]. However, we have noticed inhibition in lymphocyte activity after wet cupping practice. Ahmed and her coworker [18] have shown a marked improvement in clinical conditions of the patient, laboratory and immune cellular parameter, particularly of innate (percentage of Natural Killer, NK, cells) and adaptive cellular (Soluble Interleukin-2 Receptor, SIL-2R, concentration) immune responses, when blood-letting cupping every four weeks combined with conventional medicinal therapy was used for patients suffering from rheumatoid arthritis. In this regard, cupping therapy has similarities with acupuncture and acupressure techniques [2].

Interestingly, our study has identified, for the first time, the effect of WC on complement activity. The results revealed a significant increase in CH50 values for experimental groups relative to their controls. The precise mechanism by which WC induces complement activation is not clear at the present time, so further examination of this point may shed some light on the possible mechanisms. In this regard, Yamaguchi et al. [19] indicated that acupuncture might activate the complement system but they did not explain how this could happen. In addition, positive effectiveness of acupuncture on complement activity has been reported in rat model [20]. At a biological level; acupuncture works by stimulating or activating the immune system parameters; cellular immunity, lymphocyte count, lymphocyte transformation, T-cell CD8/CD4 ratio, phagocytic activity, serum immunoglobulin levels, salivary IgA levels, serum complement levels, natural killer cell activity and serum interferon levels [13, 21]. It seems that such effect of cupping is tangible at older ages, since no measurable effect was observed in the younger group.

The present investigation is of relatively small size, thus; future studies with larger samples, for longer duration of follow up and use of a comparison group are recommended to elucidate the long-term or short-term effects of cupping. Another limitation of the present study is the lack of a sham group. However, blinded placebo - a fake version of the procedure being studied- cupping therapy proved to be very difficult, since a reliable sham cupping intervention is presently not available. Sham cupping utilizing adhesives to keep the cups in place can usually be recognized by the subjects, even those inexperienced with cupping [9].

It might be worthwhile to examine the effectiveness of CT or combination of it with other non-pharmacological or pharmacological treatments for pain conditions.

CONCLUSION

The present study revealed the behavior of WBC and the complement activity and consequently extends and supports the belief that CT works by a mechanism that heightens the efficiency of the components of the immunity system in general. Cupping seems to improve the general physiological status in healthy subjects which happens more in people over 40. It calls for further investigation of different types of cupping to explore their effects on other parameters of cytogenetic and immunity like chromosome aberrations, micronucleus formation, % of CD8 natural killer cells, CD4 helper cells, interlukin-2 receptor and immunoglobulin's values

REFERENCES

1. Chirali I. Traditional Chinese Medicine: Cupping Therapy. 2nd edition. Philadelphia, PA, Elsevier Churchill Livingston; 2007. 615.89 C445t2007. xvii, 268 p.
2. Ullah K, Younis A, Wali M. An investigation into the effect of cupping therapy as a treatment for anterior knee pain and its potential role in health promotion. Internet J Altern Med. 2007; 4 (1). DOI: 10.5580/796.
3. Gao BB. Combination of acupuncture and wet cupping therapy on treating 92 cases with idiopathic facial palsy [in Chinese]. China J Guang Ming Chinese Med. 2010; 25:1244–1245.
4. Curtis NJ. Management of urinary tract infections: historical perspective and current strategies: Part 1-before antibiotics. J Urol. 2005; 173:21-6.
5. Michalsen A, Bock S, Lüdtke R, Rampp T, Baecker M, Bachmann J, et al. Effects of traditional cupping therapy in patients with carpal tunnel syndrome: a randomized controlled trial. J Pain. 2009; 10:601-8.
6. Hennawy M. Cupping therapy and Infertility. 2004. http://www.obgyn.net/english/pubs/features/presentations/hennawy. Vol 15/280,1. [Accessed December 2004].
7. Farhadi K, Schwebel DC, Saeb M, Choubsaz M, Mohammadi R, Ahmadi A. The effectiveness of wet-cupping for nonspecific low back pain in Iran: a randomized controlled trial. Complement Ther Med. 2009; 17:9-15.
8. Kim JI, Lee MS, Lee DH, Boddy K, Ernst E. Cupping for Treating Pain: A Systematic Review. Evid Based Complement Alter Med. 2011; 2011:467014. doi:10.1093/ecam/nep035.
9. Lauche R, Cramer H, Choi K-E, Rampp T, Saha FJ, Dobos GJ et al.The influence of a series of five dry cupping treatments on pain and mechanical thresholds in patients with chronic non-specific neck pain – a randomized controlled pilot study. BMC Complement Alter Med. 2011; 11:63.
10. Lüdtke R, Albrecht U, Stange R, Karl BU, Carstens-Stiftung, V. Brachialgia paraesthetica nocturna can be relieved by "wet cupping" - Results of a randomised pilot study. Complement Ther Med. 2006; 14:247-253.
11. Cao H, Li X, Liu J. An updated review of the efficacy of cupping therapy. PLoS One. 2012; 7: e31793. doi:10.1371/journal.pone.0031793
12. Ahmadi A, Schwebel DC, Rezaei M. The efficacy of wet-cupping in the treatment of tension and migraine headache. Am J Chin Med. 2008; 36: 37-44.
13. NIH Consensus Development Panel on Acupuncture. Acupuncture (NIH consensus conference). JAMA-J Am Med Assoc. 1998; 280(17):1518-42.
14. Al-Qashi SS, Al-Qaoud KM, J'afer M, Khalil AM. The immunocytogenetic effects of sex hormones in women undergoing in vitro fertilization treatment. Hum Exp Toxicol. 2006; 5:1-5.
15. Ahmed AE, Peter JB. Clinical utility of complement assessment. Clin Diagn Lab Immunol. 1995; 2: 509-17.
16. Niasari M, Kosari F, Ahmadi A. The effect of wet cupping on serum lipid concentrations of clinically healthy young men: a randomized controlled trial. J Altern Complement Med. 2007; 13:79-82.
17. Bilal M, Khan AR, Ahmed A, Afroz S. Partial evaluation of technique used in cupping. [original]. J Basic Appl Sci. 2011; 7: 65-8.
18. Ahmed SM, Madbouly, NM, Maklad, SS, Abu-Shady EA. Immunomodulatory effects of blood letting cupping therapy in patients with rheumatoid arthritis. Egypt J Immunol. 2005; 12:39-51.
19. Yamaguchi N, Takahashi T, Sakuma M, Sugata T, Uchikawa K, Sakaihara S. et al. Acupuncture regulates leukocyte subpopulations in human peripheral blood. Evid Based Complement Alter Med. 2007; 4: 447-53.
20. Sato T, Yu Y, Guo SY, Kasahara T, Hisamitsu T. Acupuncture simulation enhances splenic natural killer cell cytotoxicity in rats. Jpn J Physiol. 1996; 46: 131-6.
21. Donald J, Baker MD. Modern Medical Acupuncture. UMDNJ-Robert Wood Johnson Medical School. 1996; pp 1-12.