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Impact of Wet Cupping on Hematological and Inflammatory Biomarkers in Men

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أثر الحجامة الرطبة على مؤشرات الدم والالتهابات لدى الرجال

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ABSTRACT:

Background:
Wet cupping therapy, a widely accepted form of complementary and alternative medicine, has shown promise in addressing clinical symptoms associated with inflammation. This study aimed to investigate the impact of wet cupping therapy on hematological parameters and inflammatory biomarkers in men residing in Erbil.

Methods:
Experienced nurse practitioner performed wet cupping on participants in Erbil during April 26, 28, and 30, 2024. Data were collected from 21 healthy males aged 21-37 years. without takin any anti-inflammatory drugs or dietary supplements. Blood samples were taken before and six weeks after wet cupping to assess changes in hematological parameters and inflammatory markers, specifically cytokines (IL-1β, IL-2, IL-6, IL-10, and IL-12), CRP, TNF-α, GM-CSF, and G-CSF.

Results:

Analysis of complete blood counts six weeks post-cupping demonstrated significant increases in total WBCs, neutrophils, lymphocytes, monocytes, RBCs, hematocrit, and hemoglobin ($p = 0.05$ and $p = 0.01$). Conversely, the MLR, PLLR, and NLR showed significant decreases ($p = 0.05$) compared to pre-cupping levels. Furthermore, serological analysis revealed a significant reduction in the levels of cytokines IL-1β, IL-2, IL-6, IL-10, IL-12, TNF-α, GM-CSF, G-CSF, and CRP ($p = 0.05$).

Conclusion: Our findings suggest that wet cupping therapy can effectively reduce inflammatory markers and improve immune function, as evidenced by the observed changes in blood parameters and cytokine levels.

Keywords: Cupping therapy, Inflammatory markers, Hematological parameters, cytokines.

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INTRODUCTION

Cupping (Al-Hijamah) represents one of the oldest therapeutic practices, historically employed across diverse cultures and civilizations such as Chinese, Greek and Egyptian medicine, and encompassing techniques like wet cupping, which is further endorsed in Arabian tradition [1,2] and the Prophet Muhammad (Peace be upon Him) reportedly said, "The best thing you do with cupping" [3]. Wet cupping is employed for various conditions including chronic and acute infections, clinical pain, infectious diseases, and immune system disorders [4]. Studies suggest it can prevent cardiovascular diseases in both healthy individuals and those with hypertension, high cholesterol, or elevated lipids by reducing systolic blood pressure, cholesterol levels, and blood fats [5, 6].

Research indicates that wet cupping offers significant therapeutic and preventive benefits, potentially through mechanisms like reducing free radicals and boosting natural antioxidant levels [3]. It may also support immune system health, and eliminate metabolic waste, circulatory deposits, aging cells, damaged molecules, heavy metals, free radicals, toxins, autoantibodies, and poisons from blood and bodily fluids. Further, it could regenerate blood cells and stimulate neurohormones [1, 3, 7, 8, 9, 10, 11].

Several models attempt to explain cupping's effects, including theories related to nitric oxide, immune system stimulation, and blood detoxification [1]. The primary mechanisms are thought to involve passive pressure impacts that improve circulation, immune function, pain threshold, anaerobic metabolism, skin biomechanics, and reduced inflammation [12]. Wet cupping has been shown to decrease levels of inflammatory cytokines, interleukins, C-reactive protein, inflammatory growth factors, pathological growth factors, and pain-related biomolecules [2, 8, 13, 14, 15, 16, 17].

Wet cupping may lower inflammatory cytokines and interstitial fluid pressure by reducing hematocrit, hemoglobin, and erythrocyte count. It may also alleviate conditions like hypertension, obesity, cirrhosis, and fluid congestion in inflamed areas. By improving blood parameters, wet cupping may help clear old and damaged blood cells from lymphatic fluid in interstitial spaces. It may also accelerate blood flow, remove obstructions and toxins, and enhancing immunity, thereby restoring balance to organs, tissues, and cells, preventing disease, and fortify the immune system [7, 18, 19, 20, 21, 22].

This study aimed to evaluate the effects of wet cupping on hematological parameters and Immunological biomarkers. This was accomplished by comparing venous blood samples taken before and after a six-week course of wet cupping therapy in men from Erbil city.

MATERIAL AND METHODES

The study population consisted of twenty-one young healthy men aged 21 to 37 years. A certified cupping nurse practitioner in Erbil administered the wet cupping treatment over three days in April 2024. Data were collected from participants via venous blood samples, which were analyzed to assess hematological and inflammatory indices. This involved comparing blood components before and six weeks after the wet cupping intervention.

The wet cupping procedure was performed by a nurse practitioner certified by the Wisdom Academy of Integrative Sciences UK, adhering to medical and Sharia standards. Three sterile cups, each approximately 5 cm in diameter, were applied to three designated cupping sites based on prophetic medicine: two on the bilateral posterior trunk area of the thoracic spine and one in the

withers area of the midbody. The areas were cleansed and disinfected with 70% alcohol solution. Using a sterile blade, parallel incisions, 2-3 mm long and 2 mm deep, were made in the prepared skin. The cups were immediately placed, and blood began to flow within 5 minutes due to negative suction pressure created by a cupping pump. After 3-5 minutes, the cups were removed, with approximately 3-5 cm^3 of blood drained from each site. Finally, the cupping sites were covered with sterile dressings [39,40].

Venous blood samples were drawn 10 minutes before and 6 weeks after the wet cupping therapy. These samples were collected in tubes for complete blood count and chemistry analysis. The samples were immediately transported to the laboratory, and serum was separated by centrifugation at 3000 RPM for 10 minutes. A complete blood count and analysis of inflammatory markers and cytokines were then performed.

Demographic data, including name and age, were recorded for each participant. Complete blood counts, encompassing total white blood cells (WBCs), neutrophils, monocytes, lymphocytes, red blood cells (RBCs), hematocrit, and hemoglobin, were analyzed using a Mindray BC-10 instrument before and after the wet cupping therapy. Predictive hematological inflammatory parameters, namely the monocyte/lymphocyte ratio (MLR), platelet/lymphocyte ratio (PLR), and neutrophil/lymphocyte ratio (NLR), were calculated from the complete blood count data. Serum concentrations of interleukins IL-1β, IL-6, IL-12, IL-2, IL-10, tumor necrosis factor alpha, granulocyte-macrophage colony-stimulating factor (GM-CSF), and granulocyte colony-stimulating factor (G-CSF) were measured using ELISA sandwich kit specific for humans, in which the procedure has been performed according to the
kit manufacturer's protocol (Abcam, Cambridge, United Kingdom). CRP was measured using enzyme-linked immunosorbent assay (ELISA) and Beckman Coulter AU systems before and after the 6-week wet cupping period.

Data are presented in tables as mean ± SD. Statistical analysis was performed using paired t-tests in SPSS 22, with a P value ≤ 0.05 considered statistically significant.

RESULTS AND DISCUSSION

A paired t-test revealed statistically significant differences in mean ± SD of complete blood count (CBC) parameters before and after a 6-week wet cupping intervention in healthy men. Specifically, the CBC analysis demonstrated significant increases in leukocyte count, erythrocyte count, neutrophils, lymphocytes, hemoglobin level, packed cell volume (PCV), and thrombocyte count ($p \leq 0.05$, $p \leq 0.01$), as shown in Table 1. Furthermore, HCT, hematocrit, and platelet count also exhibited statistically significant increases following wet cupping therapy compared to baseline (Table 1). Notably, all observed increases remained within normal physiological ranges.

After six weeks of wet cupping, a statistically significant increase ($p = 0.0381$) was observed in the total white blood cell count in venous blood compared to pre-cupping levels (Table 1). Furthermore, complete blood count (CBC) analysis showed that the numbers of both neutrophils and lymphocytes were significantly elevated ($P = 0.00971$ and $P = 0.00786$, respectively) in venous blood post-cupping compared to pre-cupping samples. These findings align with previous research examining the impact of wet cupping on leukocyte counts in healthy individuals. CBC tests also revealed a significant difference in platelet count ($P = 0.0449$) between venous blood samples collected before and after wet cupping.

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Following wet cupping, complete blood count (CBC) analysis revealed a statistically significant elevation in red blood cell count in venous blood ($p \leq 0.0433$) compared to pre-cupping levels (Table 1). Supporting this finding, hemoglobin concentration ($p \leq 0.0392$) and red blood cell volume ($p \leq 0.0433$) also demonstrated significant increases in post-cupping venous blood compared to pre-cupping samples (Table 1), although all values remained within normal reference ranges. These findings suggest that wet cupping therapy may stimulate erythropoiesis and enhance hemoglobin production.

Table 1. Demonstrates hematologic parameters in male participants, comparing values before and after wet cupping therapy

Hematology parameters	**Pre-Wet Cupping Therapy** mean ± SD.	**Post-Wet Cupping Therapy** mean ± SD.	**P value***
WBC 10^3/µL	6.46 ± 8.205	**7**.14 ± 9.300	0.0381
RBC 10^6/µL	4.265 ± 0.732	4.435 ± 0.765	0.0433
Neutrophils 10^3/µL	57.439 ± 0.532	59.939 ± 0.532	0.0413
Lymphocytes 10^3/µL	28.893 ± 0.732	33.562 ± 0.532	0.00971
Monocytes 10^3/µL	3.21± 0.349	5.58± 0.634	0.00886
Platelets 10^3/µL	196.10 ± 402.079	207.712 ±409.549	0.0449
HB g/dL	13.124 ± 2.127	13.879 ±3.192	0.0392
HCT %	37.781 ± 0.291	42.673 ± 0.917	0.0395

* a P value ≤ 0.05 considered statistically significant

The white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HB), Platelets and hematocrit (HCT) were analyzed using a paired t-test.

Following wet cupping therapy, our research demonstrated a rise in total white blood cells, neutrophils, monocytes, lymphocytes, red blood cells, platelets, red blood cell volume, hemoglobin, and hematocrit levels, alongside a decrease in predictive hematologic inflammatory markers. These findings align with prior research indicating significant increases in red blood cell, white blood cell, hemoglobin, platelet, neutrophil, lymphocyte, and monocyte hematological parameters within normal ranges [4,25,29, 30, 31, 32, 33].

Various theories, such as the "nitric oxide theory," "immune system activation theory," and "blood detoxification theory," attempt to explain cupping therapy's mechanical and biological effects [1]. Numerous studies support the notion that wet cupping bolsters the immune system,

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stimulates immune cells, improves blood circulation, boosts lymphocyte and neutrophil activity, alleviates pain, eliminates waste and toxins, and aids in endothelial cell repair [6,5, 22, 2, 34, 4, 7, 8, 10, 11].

Current and previous data suggest that wet cupping therapy stimulates erythropoiesis, enhances hemoglobin production, and improves both the quantity and function of red blood cells and hemoglobin within tissues, ultimately increasing oxygen delivery to the body's cells and tissues. Hemoglobin (Hb) in red blood cells (RBCs) is crucial for oxygen transport, while hematocrit (PCV) reflects the volume of red blood cells in whole blood; therefore, red blood cell count and red blood cell volume are directly correlated in healthy individuals (3).

The paired t-test revealed a statistically significant decrease ($P < 0.05$ and $P < 0.01$) in monocyte/lymphocyte ratio (MLR), platelet/lymphocyte ratio (PLR), and neutrophil/lymphocyte ratio (NLR) in venous blood following wet cupping compared to pre-cupping levels. (Table 2).

Table 2. Illustrates Hematologic inflammatory markers before and after wet cupping in men.

Hematology parameters	Pre-Wet Cupping Therapy mean ± SD.	Post-Wet Cupping Therapy mean ± SD.	P value*
NLR %	1.987 ± 0.129	1.787 ± 0.122	0.0398
MLR %	0.104± 0.031	0.0801± 0.027	0.00899
PLR %	6.787 ± 0.439	6.188± 0.361	0.0274

*MLR (monocyte/lymphocyte ratio), PLR (platelet/lymphocyte ratio), and NLR (neutrophil/lymphocyte ratio) were calculated, and a paired t-test was performed, a P value ≤ 0.05 considered statistically significant

In our study, we observed results consistent with previous findings [3] regarding the utility of hematological inflammatory markers. Specifically, neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), and platelet-to-lymphocyte ratio (PLR), all easily calculated from complete blood counts, were assessed for their potential to reflect systemic inflammation [26, 27, 28]. These ratios, which express the proportion of neutrophils, monocytes, and platelets relative to lymphocytes in peripheral blood, have been linked to bacterial and viral infections, coronary heart disease, stroke, and increased mortality when elevated. Leukocytes play a vital role in the immune system, protecting the body from infectious diseases and foreign substances. Neutrophils and lymphocytes are key players in defending against bacterial and viral infections, as well as in clearing damaged cells, suggesting that this therapy may reduce systemic inflammation and exert a beneficial anti-inflammatory effect [26, 35, 37].

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In this study of 21 men, paired t-tests revealed statistically significant decreases ($P < 0.05$ and $P < 0.01$) in the levels of inflammatory cytokines IL-1β, IL-2, IL-6, IL-10, IL-12, TNF-α, GM-CSF, G-CSF, and C-reactive protein (CRP) in venous blood following wet cupping therapy (Table 3). Specifically, wet cupping demonstrated a notable effect on these inflammatory markers six weeks after a single session, as compared to pre-treatment levels (Table 3).

Table 3. Represents Cytokine and inflammatory marker profiles were assessed in male subjects to determine the impact of wet cupping therapy

inflammation markers	**Pre-Wet Cupping Therapy** mean ± SD.	**Post-Wet Cupping Therapy** mean ± SD	**P value***
IL-1β pg/mL	5.46 ± 8.205	4.14 ± 9.300	0.0431
IL-2 pg/mL	4.46 ± 0.732	3.75 ± 0.761	0.0389
IL-6 pg/mL	11.23±5.36	9.34±4.23	0.00798
IL-10 pg/mL	8.67 ± 0.732	7.28 ± 0.532	0.0291
IL-12 pg/mL	6.21± 0.349	4.58± 0.634	0.00923
TNF-α pg/mL	4.36 ± 2.72	3.23 ±1.23	0.0457
GM-CSF pg/mL	7.59 ± 6.73	5.75 ±7.38	0.0483
G-CSF pg/mL	6.85 ± 3.93	4.68 ±4.81	0.00639
CRP mg/mL	2.85 ± 1.76	1.18 ±1.21	0.00869

*The statistical analysis used was a paired t-test. Abbreviations: CRP (C-reactive protein), IL (interleukin), TNF-α (tumor necrosis factor alpha), GM-CSF (granulocyte-macrophage colony-stimulating factor), and G-CSF (granulocyte colony-stimulating factor), a P value ≤ 0.05 considered statistically significant.

In this study, wet cupping therapy in male subjects significantly reduced ($P < 0.05$ and $P < 0.01$) the levels of several inflammatory cytokines and C-reactive protein (CRP) in venous blood. Specifically, significant decreases were observed in IL-1β, IL-2, IL-6, IL-10, IL-12, TNF-α, GM-CSF, and G-CSF (Table 3). We evaluated the effect of a single wet cupping session on these inflammatory markers in 21 healthy men, and found that the procedure exerted a significant impact on them six weeks after its application (Table 3). These findings align with theories suggesting that cupping's anti-inflammatory effects stem from the removal of toxins and/or the activation and stimulation of immune responses [1, 25]. Consistent with Ekrâmi *et al*. [25], who reported a decrease in IL-6 and TNF-α in athletes after wet cupping, our results, along with those of other studies [3, 23, 30], demonstrate a positive impact of cupping therapy on reducing inflammatory cytokines and molecular biological markers. This suggests that wet cupping enhances the body's

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immune system and detoxification processes by eliminating damaged cells and foreign substances. Moreover, Abdelfattah *et al*. [3] found that wet cupping significantly increased total white blood cell count and complement protein activity in healthy men, indicating a stimulatory effect on both cellular and humoral immunity. Furthermore, Ahmed et al. demonstrated that cupping therapy can modulate immune system proteins, leading to improvements in clinical symptoms, laboratory indicators (ESR, CRP, rheumatoid factor), immunological markers (NK cell ratio), and cytokine receptors (IL-2) in rheumatoid arthritis patients [37].

CONCLUSIONS

The findings of our study demonstrate that cupping therapy significantly reduces circulating inflammatory and pathological physiological markers, including cytokines, chemokines, and other inflammatory proteins and increases the number, effectiveness and activity of immune cells and immune-related biological molecules, and activating their functions in the body. These play a key role in reducing inflammation and disease symptoms, alleviating pain and accelerating the recovery process, thus confirming the safety and efficacy of wet cupping in managing pain and infectious and physiological diseases. The wet cupping therapy has a significant positive impact on improving and enhancing the efficiency and capabilities of cellular and humoral immunity, and reducing levels of inflammatory and pathological physiological indicators in the body. The wet cupping therapy helps to remove excess fluids, toxins and damaged and aged cells from the blood and boosts the immune system, and provides a physiological and immunological understanding of the positive effects and health benefits of wet cupping therapy.

Future research using a larger sample size is required to confirm the results of the current study and to explain mechanism(s) fundamental such beneficial effects of cupping therapy and its potential application in various health and disease conditions.

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Conflict of interests.

There are non-conflicts of interest.

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الخلاصة:

تعد العلاج بالحجامة Cupping Therapy من أحد أهم استخدامات الطب التكميلي و البديل Complementary and Alternative Medicine التي حظيت بالقبول الكبير في جميع انحاء العالم, وتلعب السيتوكينات الالتهابية Inflammatory cytokines دورًا مهمًا في الأعراض السريرية المرضية. صممت دراستنا للكشف والتحقق من أثر استخدام العلاج بالحجامة الرطبة على مؤشرات الدم والتغيرات في علامات الالتهابات لدى الرجال الاصحاء في مدينة أربيل.

تمت عملية الحجامة الرطبة من قبل ممرض ممارس في اربيل صباح يوم 17 و19 و21 من شوال 1445 هجري الموافق 26 و 28 و30 نيسان 2024 ميلادي. وتم الحصول على البيانات من 21 مشاركًا متطوعاً من الذكور الأصحاء الذين تتراوح أعمارهم بين 21 و 37 عامًا, وتم تقييم مؤشرات الدم والالتهابات كالسيتوكينات (IL-1β و IL-2 و IL-6 و IL-10 و IL-12) و و TNF-α , وبروتين سي التفاعلي CRP ، وعامل تحفيز مستعمرة الخلايا المحببة البلعمية (GM-CSF) وعامل تحفيز الخلايا المحببة (G-CSF) من خلال مقارنة مكونات الدم الوريدي قبل وبعد ستة أسابيع من الحجامة الرطبة.

أظهرت نتائج الدراسة لتحليل تعداد الدم الكامل CBC لعينات الدم الوريدية بعد ستة أسابيع من الحجامة الرطبة زيادة معنوية في قيم إجمالي خلايا الدم البيضاء WBCs والخلايا المتعادلة والخلايا الليمفاوية والخلايا الوحيدة وخلايا الدم الحمراء RBCs والهيماتوكرايت والهيموجلوبين مقارنة مع أخذ عينات من الدم الوريدي قبل الحجامة. وأظهرت تحليل المعايير الدم الالتهابية التنبؤية بعد الحجامة انخفاضًا ملحوظًا في نسبة الخلايا الوحيدة/الخلايا اللمفاوية MLR ونسبة الصفائح الدموية/الخلايا اللمفاوية PLR ونسبة العدلات/الخلايا الليمفاوية NLR. وكشفت الاختبار المصلي لعينات الدم الوريدية بعد 6 اسابيع من الحجامة انخفاضا ملحوظا ($P < 0.001$, $P < 0.005$) في سايتوكينات IL-1β، و IL-6، و IL-12، و IL-2، و IL-10، وكيموكينات TNF-α، و GM-CSF، و G-CSF، و بروتين سي التفاعلي CRP .

نستنتج من هذه الدراسة أن الحجامة الرطبة تعمل على تقليل مستويات مؤشرات الالتهابية والفسيولوجية المرضية في الدم كالسيتوكينات والكيموكينات والبروتينات الالتهابية وزيادة اعداد وفعالية ونشاط الخلايا المناعية والجزيئات الحيوية المناعية و تفعيل أليات عملها في الجسم.

الكلمات المفتاحية: الحجامة, الطب البديل, مؤشرات الدم, المعايير الالتهابية, سايتوكينات.

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