https://doi.org/ 10.33472/AFJBS.6.Si3.2024.82-98

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Influence of Cupping Therapy on Immune Systems in Post COVID-19 Patients

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Article History

Volume 6,Issue Si3, 2024

Received:21 Mar 2024

Accepted : 08 May 202

doi: 10.33472/AFJBS.6.Si3.2024.82-98

Abstract

Background: Worldwide, 1,914,916 people got infection with COVID-19 in the middle of April 2020, with 123, 010 losses and 501,758 survived, according to the World Health Organization (WHO). Researchers have discovered that after a coronavirus infection, certain signaling pathways can impact how the immune system's cells work. These pathways include the toll-like receptor as well as the retinoic acid-inducible gene-I-like receptor signaling pathways. As a result, these cells can produce a variety of cytokines associated with the 17-receptor, which can further impair both the innate in addition to adaptive immune systems. **PURPOSE:** to examine the impact of dry cupping therapy on T-Lymphocyte, IL-6, IgA, IgM and IgG in patients with post-COVID-19. **DESIGN**: Patients were assigned randomly into two equal groups by randomized controlled trial. **SETTING**: Patients with post Covid-19 from Outpatient Clinic Shobra General Hospital, Cairo governate, Ministry of Health,Egypt. **SUBJECTS**: seventy- six patients (21 to 66 years old) previously infected with SARS-CoV-2. **METHODS**: Over the course of eight weeks, patients were divided into two equal groups utilizing a computer-generated block randomization program. Group "A" received dry cupping therapy as well as traditional medical treatment, while group "B" received the same traditional medical treatment program. The patients' pre- and post-treatment evaluations were performed using body weight and height scale for measuring weight and height then calculating BMI, Flow Cytometry device for detecting physical and chemical characteristics of cells or particles, Automatic biochemistry analyzer for cytokine detection and Semi- Automatic protein analyzer for IgA, IgM, IgG detection. **RESULTS**: mixed design MANOVA comparisons showed that all variables in both groups differed significantly between before- and following treatment, with a p-value less than 0.05. After the therapy, the analysis revealed that the study group had statistically significant improvements over the control group (p-value <0.05) when comparing the two groups. **CONCLUSION:** Dry cupping has a positive effect on the immune system, and inflammatory markers in post COVID-19 patient.

.**Keywords**: Cupping Therapy, Immune system, Covid-19, Post covid-19

Introduction

The city of Wuhan in China's province of Hubei has seen an upward trend in serious pneumonia infections since December 2019, and the illness has subsequently spread fast across the country and even to other nations (Lu et al., 2020). The novel Beta coronavirus that caused severe acute respiratory syndrome (SARS) and pneumonia (COVID-19) has been designated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Huang et al., 2020). China has managed to stem the spread of the epidemic through a combination of medical treatment and preventative measures (Tang et al., 2020).

Around the world, 1,914,916 individuals were infected by the disease in mid-April 2020, including 123, 010 people dying and 501,758 people making a full recovery, according to the WHO (Balachandar et al., 2020). Patients can get recover following an infection, but they may still have to deal with the consequences for a while. So, it is critical to learn what happens to individuals who recover from COVID-19 and whether they are more likely to have other harmful conditions by doing longitudinal studies. To properly manage the physical and mental health of these patients, it is important to follow up with them after they have recovered and do thorough evaluations (Balachandar et al., 2020).

Research has demonstrated that when coronavirus infections occur, certain signaling pathways can impact the activity of immune cells. These pathways include the toll-like receptor as well as the retinoic acid-inducible gene-I-like receptor signaling channels. As a result, a plethora of 17-related cytokines are produced, which can exacerbate adaptive as well as innate disorders of the immune system (Kinder and Thiel, 2014). The fast spread of COVID-19 and the absence of specific treatments highlight the significant issue of our current understanding of the virus's effects on the immune system (Wen et al., 2020).

A variety of terms were used to describe conditions that occurred after the COVID-19 pandemic: post-COVID-19 syndrome, chronic COVID-19, extended COVID-19, long-term consequences of COVID-19, in addition to post-acute sequelae of SARS-COV-2 infection. When an individual acquires COVID-19, these symptoms and others like them show that their health cannot return back to normal. For an illness to be considered post-COVID-19, it must have occurred for a minimum of four weeks following the start of the virus, have symptoms that have persisted for at least two months, and not be attributed to any other medical condition. The post-COVID-19 syndrome is common among children (Pierce et al., 2020).

Two weeks following a patient's recovery from a COVID-19 infection, their peripheral blood was examined for cytokines and lymphocyte subsets. Based on their findings, we propose reevaluating the present standards for hospital discharge (Hasichaolu et al., 2020) to determine why some patients became ill following their discharge.

In the case of a respiratory infection, lymphocytes are essential for the removal of viruses. Studies have shown that SARS-CoV-2 infections can disrupt the levels of certain types of lymphocytes. For example, patients infected with COVID-19 had lower total counts of CD3+ T cells, CD4+ T cells, CD8+ T cells, CD19+ B cells, as well as CD56+ NK cells (Huang et al., 2020 and Lu et al., 2020).

This information points to the possibility that the coronavirus may suppress the body's cellular immunity by destroying a large number of immune cells. In addition, even when the condition improved, the total lymphocyte levels remained below normal (Hasichaolu et al., 2020). The findings are in line with what was previously published, which showed that COVID-19 individuals' peripheral blood NK

as well as T cell counts dropped during both the initial and final phases of recovery (Wen et al., 2020). According to prior research, cytokine storms are a major factor in severe cases of COVID-19 (Zheng et al., 2020).

After attaching to alveolar epithelial cells, SARS-CoV-2 triggers the body's immune systems, both adaptive and innate, which in turn release a plethora of cytokines. Nevertheless, cytokine levels were substantially greater in COVID-19 patients who made a full recovery. According to Hasichaolu et al. (2020), patients who recover from SARS-CoV-2 infection will have a better ability to fight the virus after a specific amount of time has passed. After that, they will start to exhibit signs of a relatively active immune response.

There is currently no medical procedure that can remove harmful compounds from the blood as well as interstitial fluids, which are both involved in disease development. There is global and cultural acceptance of CAM or complementary and alternative medicine. CAM refers to a wide range of complementary and alternative medicine practices that can be used in combination with traditional medicine to alleviate symptoms (Salah et al., 2020).

A wide variety of medical issues can be alleviated by CAM treatments. They offer a platform that is crucial for an individual's well-being and health. It is crucial to investigate their scientific and biological depth because these are commonly employed to improve people's health. According to Salah et al. (2020), the absence of a physiological mechanism for the excretion of excess undesirable compounds in blood and interstitial fluids can lead to many diseases by disrupting blood chemistry as well as physiological homeostasis.

The use of cupping as a therapeutic modality has a history in TCM, which dates back at least two thousand years. There are many variations of cupping techniques, including retained, flash, moving, moist, medicinal, as well as needling cupping. The material of the cup itself can range from bamboo to glass to pottery. Although the exact process by which cupping therapy works is still unclear, several studies have linked its therapeutic effects to the hyperemia or hemostasis that takes place when cups are placed on specific acupoints on the skin (Cao et al., 2012).

All the medical diseases listed above may potentially benefit from cupping therapy. The combination of pharmaceutical and excretory treatments, such as cupping therapy, may improve therapeutic outcomes and patient advantages. Reproducing cupping results requires a detailed description of the procedure. The most reliable method is mechanical suction using a balloon. Salah et al. (2020) found that beginners require ten to twenty attempts before they can achieve steady pressures.

A new coronavirus, SARSCoV-2, is responsible for the COVID-19 pandemic. ARDS is caused by this highly infectious virus, which infects both the upper and lower respiratory tracts. However, in certain individuals with preexisting conditions such as diabetes, immunodeficiency diseases, hypertension, chronic kidney failure, or cardiovascular illness, the mortality rate from COVID-19 may be higher than the overall 3.4% (Hasichaolu et al., 2020).

According to Griffoni et al. (2012), the immune system's reaction to SARS-CoV-2 varies depending on factors such as the host's gender, age, dietary habits, physical state, as well as genetics (HLA genes). These factors interact to determine the development of COVID-19. Innate immunity as well as adaptive immunity are the two parts of the immune system's reaction. The 1st one is made up of both physical and chemical restrictions, and it involves cells like neutrophils, NKs, and macrophages as well as chemicals like cytokines, interleukins, NO, as well as O2-. T cells (TCD4 and TCD8 +) and B cells (and the antibodies and cytokines they produce)

are the targets of the 2nd one, which has a mechanism of action (Janeway and Medzhitov, 2012).

The objective of post-COVID-19 rehabilitation is to alleviate respiratory symptoms, maintain function, and lessen disability and consequences. For a psychological level, it has a beneficial impact by alleviating anxiety and despair (Agostini et al., 2021). People dealing with the effects of the COVID-19 pandemic require support in coping with their symptoms, particularly fatigue, and in safely engaging in physical activity for its possible health advantages (Humphreys et al., 2021).

Additional subtypes of the adaptive immune reaction include cellular immunity, which is mediated by cells like lymphocytes and macrophages, as well as humeral immunity, which is mediated by antibodies. Sustained hyper-inflammatory reactions for greater than 14 days among individuals' post-recovery indicates the necessity for medical, physical, as well as psychosocial care (Hasichaolu et al., 2020).

Clinical trials of cupping, a non-pharmacological method of treating respiratory symptoms like coughing and shortness of breath in the COVID-19 pandemic, have shown promising results. In cases of pneumonia, acute pulmonary injury, and ARDS, there is evidence that cupping therapy significantly improves survival, increases the rate of hospital discharge, and increases the rate of cure (Xu et al., 2020).

Based on what is known, there is a dearth of data regarding the effects of dry cupping upon T-lymphocytes, serum cytokines, and immunoglobulin A, M, as well as G levels in patients following COVID-19. So, after COVID-19, this study set out to examine how cupping therapy affected T-lymphocytes, serum cytokines, and immunoglobulin A, M, and G levels. Physiotherapists working in both public and private hospitals and clinics will find this material useful. One possible addition to physical therapy for patients recovering from COVID-19 is cupping therapy, which inhibits immunopathogenesis by lowering serum levels of autoantibodies, inflammatory markers, as well as serum ferritin, an important player in autoimmunity. The technique is also relatively easy and safe to implement.

Subjects

This study was conducted to detect the impact of dry cupping therapy on immune system in post-COVID-19 patients from March to June 2022 at physiotherapy out patient clinic and laboratory department of Shobra General Hospital, Cairo, Egypt.

Ethical approval and registration consideration:

The research followed the guidelines laid out in the Helisiniki declaration, which is the World Medical Association's code of ethics. The University of Cairo's Faculty of Physical Therapy's Ethical Committee gave their approval to the research procedure (No.P.T.REC/012/003554) (Appendix I). Prior to the beginning of the study procedures, the patient's involvement was allowed by a written consent form that had been completed by both the parent or legal guardian (Appendix II).

I) Design of the study:

Randomized Controlled Trail as patients were assigned randomly into two equal groups according to inclusion and exclusion criteria.

II) Subjects:

Seventy-six male and female patients with a history of SARS-CoV-2 infection participated in this study. (including 37 men and 39 women) took part in the research. They were chosen from the Shobra General Hospital Department in Cairo, Egypt. Based on the sample size calculation utilizing the G-power test (effect size = 0.66,

power = 0.8, α = 0.05), all patients who had previously been treated in the hospital were discharged when their physical indices met the discharge standards set by the national "Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia." **(Zhao et al., 2020).**

Sample size calculation:

Patient were selected randomly and distributed in two groups by computer generation. Utilizing G*Power (version 3.0.10), the sample size was determined. The F-test MANOVA was chosen for both the within- and between-interaction effects. With α=0.05, G*Power = 80%, and effect size = 0.66, a minimum of 38 individuals per group and an overall of 76 subjects were needed for the obtained sample size **(fig.7).**

Subject's selection

76 patients of both genders were previously infected with SARS-CoV-2 were enrolled in this study. Patients were randomized allocated into two equivalent groups, twenty patients in every group and they were recruited at physiotherapy outpatient clinic and laboratory department of Shobra General Hospital, Cairo, Egypt. Discharge criteria according to the national "Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia" were used to determine whether a patient could be released from the hospital after receiving treatment **(Zhao et al., 2020).**

Seventy-six patients from both sexes aged from 21 to 66 years with a BMI between 25 to 29.9 kg/m² diagnosed as two-week post recovery from covid-19 who had lower absolute number of T-Lymphocyte, elevated level of cytokine and lower level of immunoglobulin IgA, IgM and IgG **(Hasichaolu et al., 2020)**. During the eligibility assessment, ten patients were excluded because six didn't fulfill the inclusion criteria and four refused to participate in the study because of long period of exercise program.

Randomization Method:

We used a computer-generated block randomization method that you can find at http://www.randomization.com/ to carry out the randomization. A 1:1:1 allocation ratio was used to randomly assign participants to blocks of 6 or 9. Sealed, opaque envelopes were numbered consecutively for concealed allocation. An impartial researcher who had no hand in participant selection, data collecting, or administration of any kind of treatment administered the randomization.

Criteria for selection:

The criteria for patient's selection were classified into two main categories:

(A) Inclusion criteria:

Patients to be admitted to the study should have the following inclusive criteria:

All recruited subjects including two-week post recovery from covid-19 who have lower absolute number of T-Lymphocyte, elevated level of cytokine and lower level of immunoglobulin IgA, IgM and IgG **(Hasichaolu et al., 2020);** All participants were clinically and medically stable; Age ranged from 21 to 66 years **(Hasichaolu et al., 2020);** The BMI of all patients was from 25 to 29.9 kg/m^2.

(B) Exclusion criteria:

If a potential subject met any of the following conditions, they were not included: Medical conditions such as a history of infections, whether acute or chronic, problems with the liver or blood, problems with the urinary system, problems with nutrition and metabolism, rheumatism, hormonal imbalances, or problems with the cardiovascular system Muscle injury; high blood pressure; being pregnant.

Further, if they fulfilled any of the following testing criteria:

Antibodies against hepatitis C and human immunodeficiency virus antibodies; The levels of creatinine, creatine kinase, uric acid, glucose, and C-reactive protein should all be above 120 µmol/L, 500 µU/L, 475 µmol/L, 7.0 µmol/L, and 12.0 µm, respectively.

They were assigned into two groups

- **Group A (Experimental group):** This group involved 38 patients who was given dry cupping therapy as well as traditional medical treatment.
- **Group B (Control group):** This group involved 38 patients who was given the identical traditional medical treatment of group (A).

Flow chart of the study:

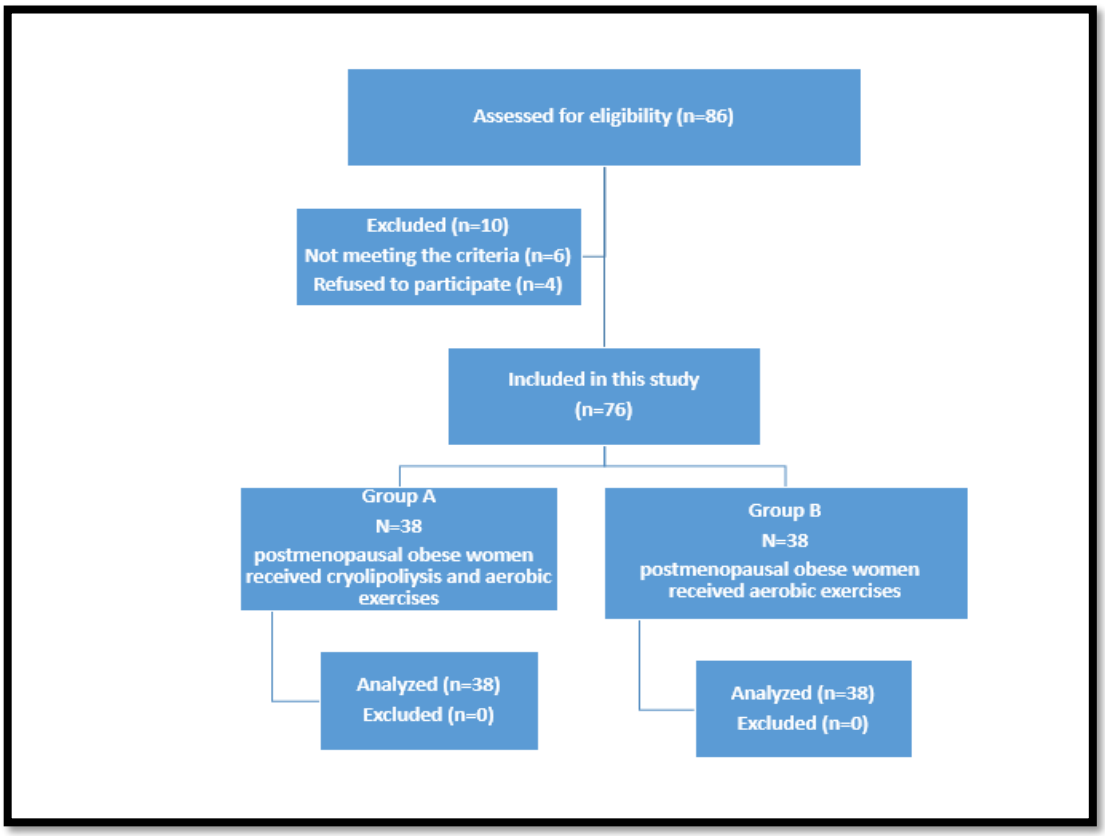

Fig.(1): **Criteria of Eligibility.**

III) Instrumentations:

A- Instruments for assessment:

1- Body weight and height scale:

It is used for measurement of height and weight for BMI calculation **(Fig.9).**

2- Flow cytometry:

It is a laser-based method for identifying and evaluating the physical and chemical properties of particles or cells. It is most frequently used to assess peripheral blood, bone marrow, and other bodily fluids. It's also a laboratory test meant to examine particle or cell properties. A sample of cells or fragments has been suspended in fluid and administered into a flow cytometer machine throughout the procedure. A computer is capable of analyzing and processing about 10,000 cells in less than a minute (Fig.10).

Particularly, flow cytometry is employed in research for several reasons, like Counting cells; Sorting cells; Identifying the characteristics and function of cells;

Identifying microorganisms, including bacteria, fungus, or yeast; Identifying biomarkers (features that signify regular function); Blood as well as bone marrow cancer diagnosis and possible treatment **(Ornatsky et al., 2010)**

3- Automatic biochemistry analyzer (Kenza 240 TX / ISE):

It is valid and reliable automated biochemistry analyzer (Compact and Convenient). Bio Lab. France makes a chemical autoanalyzer that can identify cytokines. Inductively Coupled Plasma-Optical Emission Spectrometry (OPTIMA 2100DV, Perkin Elmer) was used to determine the iron as well as lead concentrations in the blood **(Salh et al., 2014).** It offers a real throughput of 240 tests per hour and is one of the analysers starting the fastest. Its new barcode reader technology (video-camera) **(Fig.11)** and its new range of dedicated reagents in bar-coded containers make it a fully automated solution **(Tanuriria and Mushawwir, 2020).**

Specifications: Manufacture: BIOLABO; Model No.: Kenza 240 TX; Country of Origin: France.

4- Semi-automatic protein analyzer (Genrui PA50):

When you need a backup model for a specific protein, like immunoglobulins IgA, IgM, or IgG, PA50 are small and easy to use. This machine is perfect for small labs looking to enhance their laboratory analysis capabilities. It has state-of-the-art nephelometry technology, user-friendly software, standard operation, and sustaining that does not involve liquid flow.

Specifications:

The following features are available: a 5.6-inch touch screen, an incubator temperature of 37±0.1o C, the ability to store 100,000 samples, and a throughput of up to 40 samples per hour or 90-210 seconds each sample. Marked by the CE, FDA, and NGSP, this magnetic card calibration is guaranteed. Originating from: Switzerland.

B- Instruments and modalities for treatment:

1- Dry cupping therapy:

According to Uddin et al. (2016), this approach is both valid and reliable, and it can be made from a lot of different materials. Some examples of these materials are clay, bamboo, and glass. It is recommended to use disposable cups because non-disposable cups must undergo extensive sterilization as well as disinfection processes before they can be reused. The size of the cups, as shown in Figure 13, depends on their intended use (Moura et al., 2018).

Warm, negative pressure is applied through cupping, which is believed to alter the permeability of local capillaries, leading to their rupture and the release of histamines, serotonin, and various other chemicals. It gets the body's defenses ready for fighting disease by triggering the immune system as well as the autonomic nervous system **(Liu et al., 2022).**

IV) Procedures of the study:

A- assessment procedures: Before centrifugation at 2000-3000 rpm for 5 minutes to extract serum, two milliliters (mL) of venous blood from each participant was transferred to EDTA anticoagulation tubes, as well as four mL of venous blood was transferred to serum-separating tubes containing gold tops, as well as separating gel. We discarded samples that showed signs of hemolysis, jaundice, or elevated lipid levels **(Hasichaolu et al., 2020).**

1-Assesment of BMI:

All patients in both groups had been assessed by the weight and height scale to detect the BMI according this equation weight/hight^2. They were asked to wear thin

layer of clothes. After that they were asked to stand on the right place on the standing board with both hand in front of them with fisted hand beside their body, and looking forward, then stand for while till the analogue stop moving. Then the height was measured by asking the patient to stand erect on the stand board without shoes, feet straight ahead, face was looked forward and the stadiometer was lowered so that the hair pressed down, and the height was scored in meter^2.

2-Detection and analyzation the chemical and physical characteristics of cells or particles:

The flow cytometry system was used to measure it after a blood sample was injected with a suspension. After aligning the cells within a single file line, they were subjected to the laser beam. After that, the cells were sorted and counted. A computer was used to store the data, and it was then shown using a dot plot or histogram.

Lymphocyte subset detection:

Each sample was assigned a unique label and a single flow tube was created. Ten microliters (μL) of the CD4-RD1/CD8-ECD/CD3-PC5 monoclonal antibody has been added to tube A. The appropriate flow tubes were then filled with 100 μL of EDTA-anti coagulated blood from each patient, vortexed, and left to incubate at 23-28 degrees Celsius in darkness for 20 minutes. The next step was to add 1 mL of lysing solution to each tube. The samples were spun at 200 × g in a low-speed centrifuge for 5 minutes after being incubated at room temperature in the dark for an extra 20 minutes. The supernatant was then removed, and 2 mL of PBS was transferred to the tube. The samples received another centrifugation at 200 × g for 5 minutes. The liquid above was removed by aspiration, and 500 μL of PBS was then added. After that, a DxFLEX flow cytometry device bio lap diagnostic was used to evaluate the samples using fluorescence-labeled flow cytometry **(Morales et al., 2017).**

Cytokine detection:

To obtain serum for examination, venous blood samples were centrifuged at 2000-4000 rpm for 20 minutes in tubes with separating gel. Following the manufacturer's directions, IL-6 was identified using microsphere flow immunofluorescence. Once the blood samples and their matching flow tubes were assigned numbers, the microsphere mixture that had been collected was spun at 200 × g in a low-speed centrifuge for 5 minutes, and the liquid above was delicately removed. After adding a volume of microsphere buffer equal to that of the supernatant, the samples were properly mixed using a whirlpool before being incubated in darkness for 30 minutes. The following step was to combine 25 εL of the previously incubated microsphere mixture, 25 εL of centrifuged serum, as well as 25 εL of fluorescent reagent within the appropriate flow tube, after which all of the tubes were gently mixed. One milliliter of PBS was supplied to every flow tube after incubation at 23–28 degrees Celsius for 2.5 hours in darkness. The supernatant was carefully removed after 5 minutes of centrifugation at 200 × g, and 100 μL of PBS was then added to the flow tube. After that, a calibrated flow cytometer was used for detecting fluorescent molecules on the samples **(Liu et al., 2020).**

IgA, IgM and IgG detection:

Venous blood sample were obtained, after an overnight fast, between 0800-0900 h. in vacationer tubes. Blood samples were centrifuged at 2000g for 10 minutes and serum samples obtained were analyzed immediately **(Bonilla and Oettgen, 2020).**

B- Procedures for treatment:

The following was used to randomly assign 76 patients who fulfilled the aforementioned criteria to one of two groups, A or B, with an equal number of participants in each:

Group A (Experimental group):

This group included 38 patients who received dry cupping therapy one session per week for 8 weeks with a six-day break in between treatments and eight minutes of skin-contacting cup application in addition to traditional medical treatment for 8 weeks in the form of **(Fowler et al., 2021):**

Daily vitamin D intake for adults younger than 70 years old should be 600 international units (IU); Vitamin C: 200 milligrams per day is the suggested daily allowance; Noxaparin, a low-molecular-weight heparin, is the medicine of choice for anticoagulation (blood thinners). The recommended dosage is 30 mg, administered subcutaneously once each 12 hours.

A- Instructions before applying dry cupping therapy (Fan et al., 2016):

Proper counseling should be done to the patient about the procedure and marks, take consent form if required; Surface should be cleaned or disinfected before cupping; Use disposable cupping glasses for each patient to avoid disinfection procedure; Do not shave the site where cupping therapy would be conducted within four hours of the treatment; The therapist does not mind the hair, and the skin would benefit from this; Instruct male patient to shave the region where cupping applied four days before treatment; Check of the patient's skin if it is sensitive or not; Check for wounds, cracks, and higher than the normal local temperature; After the process, the therapist should apply antiseptic cream or moisturizer, this helps prevent possible infections; Recover from sunburns when patient undergoes cupping therapy, The temperature at the treatment site seems higher than normal; Sunburned skin will not be comfortable with the added heat or the cup; Apply calming aloe vera to the sunburns, then allow for the skin to heal before scheduling a second cupping session.

B- Dry cupping therapy procedures (Silva et al., 2023):

Subjects were instructed to assume the crock line position during the technique's administration and given information regarding the following: the sensation of initial suction, the possibility of bruising at the application site, any side effects that could be reported, and the option to be referred to a specialist if needed; All of the treatments occurred in an air-conditioned room at the physical therapy facility. The cupping method required a hand-held suction pump in addition to five acrylic cups; During cupping therapy, therapist placed a cup on patient's skin on the five points for immune system; This created suction using rubber pumps; The air in it forms a vacuum, which drawled the muscle and skin upwards into it; The patient's skin might turn red since the blood vessels are responding to pressure change; The cups were secured in place using adhesive tapes to prevent them from falling.

C- post-cupping after care:

To achieve the best possible outcome of cupping therapy, it’s critical to adhere to a few guidelines **(Fowler et al., 2021):**

Stay hydrated by drinking plenty of water. This will help the lymphatic system remove the cellular waste products released by the organs and tissues during cupping therapy. Those who undergo this treatment may experience flu-like symptoms (headache, body ache, etc.), unusual fatigue, or other similar symptoms. This is a temporary immunological response to the treatment-induced cellular debris; Keep warm try covering the sites where cupping therapy was done.

D- Points for immune system (Subadi et al., 2014):

The stomach (36) (ST36) three centimeters underneath the kneecap on the outside of the tibia and fibula**.** Positioned four finger widths below the knee cap, near the outer edge of the shin bone, ST36 is an often-used point for gastrointestinal distress, nausea, vomiting, stress, as well as exhaustion.

The spleen (6) (SP6) behind either tibia or fibula and just above the ankle**.** SP6 can be found on the lower calf, which is above the ankle, on the posterior side of the shinbone. It's approximately four finger widths higher than the inside of the ankle.

The lung (7) (L7) on the inside of the wrist**.** Locate L7 near the styloid process of the radius bone, that is the forearm bone that ends nearest to the thumb, and it is located just proximal, or more closely to the torso.

The large intestine (11) (LI11) on the outside of the arm**.** LI11 lies along the lateral aspect of the forearm and is a super empowering point for both physical and emotional exhaustion. The LI4 is situated on the palm side of the hand, deep inside the space between the webs of the thumb and the index finger. It is thought that the LI4 point triggers labor. It has a number of potential problem-solving functions, including reducing pain and boosting immunity.

Group B (Controlled group):

This group involved 38 patients who was given the same traditional medical treatment of group (A) for 8 weeks.

Data collection and statistical analysis

The data was tested for normality utilizing the Shapiro-Wilk test before analysis. The homogeneity of variances among groups was tested using Levene's test. A homogeneity of variance was observed, and the data followed a normal distribution.

A. **Descriptive statistics**: For every parameter, we calculated the group means and standard deviations.
   - Mean (X) = summation of x / number of x.
   - Standard deviation (SD) = root square of variance.

B. **Interferential Statistics:**

To compare the groups based on age and body mass index, we used an independent t test; to compare the groups based on sex, we used chi-squared tests. The mean values of T-cells, IL-6, IgA, IgM, and IgG were analyzed using a mixed MANOVA that compared the effects of time (before versus after), treatment (among groups), and the interaction among the two. Subsequent multiple comparisons were corrected using Bonferroni; All statistical tests were conducted with a significance level of $p < 0.05$, and the statistical analysis was carried out using SPSS version 25 for Windows.

Statistical analysis

When comparing groups based on age and BMI, an independent t-test was employed; when comparing groups based on gender distribution, a chi-squared test was employed. We used the Shapiro-Wilk test to make sure the data was normally distributed. The homogeneity of variances among groups was tested using Levene's test. To assess the effects on T-cells, IL-6, IgA, IgM, and IgG within and across groups, a mixed MANOVA was conducted. For the subsequent multiple comparison, Bonferroni corrections were applied. All statistical tests were set to have a significance level of $p < 0.05$. Our entire statistical analysis was carried out using SPSS version 25 for Windows, which is developed and maintained by IBM SPSS in Chicago, IL, USA.

- Results

- *Subject characteristics*:

The subjects in both Group A and Group B are shown in Table (1). The distribution of ages, BMI, and sexes did not differ significantly ($p > 0.05$) among the groups .

Table 1. Comparison of subject characteristics between group A and B:

	Group A	Group B	Statistics	p-value
Age, mean ± SD (years)	41.39 ± 8.92	40.68 ± 11.37	(t = 0.30)	0.76
BMI, mean ± SD (years)	28.47 ± 1.09	28.65 ± 1.12	(t = -0.71)	0.47
Sex, n (%)				
Female	18 (47%)	21 (55%)	(χ^2 = 0.47)	0.49
Male	20 (53%)	17 (45%)		

SD, Standard deviation; t, unpaired t value; χ^2, Chi squared value; p value, Level of significance.

<u>Impact of treatment on T-cells, IL-6, IgA, IgM and IgG:</u>

Time and treatment interacted significantly ($F = 201.04$, $p = 0.001$, partial eta squared = 0.93, as shown by mixed MANOVA). $F= 1059.97$, $p = 0.001$, partial eta squared = 0.98) indicates that time is a significant main effect. $F=119.12$, $p = 0.001$, partial eta squared = 0.89) showed that the treatment had a substantial main impact.

Within group comparison

After treatment, compared to before treatment, group A and B had a significantly higher number of T-cells and a significantly lower level of IL-6 ($p < 0.001$). Group A's T-cell and IL-6 percentage changes were 62.07% and 74.49%, correspondingly; group B's numbers were 29.17%, 19.1%, and 32.09%, respectively. (Table 2).

The levels of IgA, IgM, and IgG in both groups A and B were significantly lower after therapy when compared to before treatment ($p < 0.001$). Group B's percentage changes for IgA, IgM, and IgG were 24.91%, 22.98%, and 13.42%, correspondingly, while group A's percentage changes were 60.57, 53.94%, and 50.23%, respectively. (Table 3).

Between group comparison

Before treatment, there was no statistically significant difference among the groups ($p > 0.05$). After treatment, group A had significantly more T-cells and significantly lower levels of IL-6, IgA, IgM, and IgG compared to group B ($p < 0.001$). (Table 2-3).

Table 2. Mean T-cells and IL-6 before and after treatment of group A and B:

	Group A	Group B		
	Mean ±SD	**Mean ±SD**	**MD**	**p value**
T-cells (ng/dl)				
Pre treatment	635.53 ± 148.13	630.79 ± 135.21	4.74	0.88
Post treatment	1030 ± 121.43	814.79 ± 93.05	215.21	0.001
MD	-394.47	-184		
% of change	62.07	29.17		
	p = 0.001	***p = 0.001***		
IL-6 (pg/ml)				
Pre treatment	99.34 ± 22.61	95.14 ± 21.01	4.2	0.40
Post treatment	25.34 ± 4.92	64.61 ± 6.68	-39.27	0.001
MD	74	30.53		
% of change	74.49	32.09		
	p = 0.001	***p = 0.001***		

SD, Standard deviation; MD, Mean difference; p value, Probability value.

Table 3. Mean IgA, IgM and IgG before and after treatment of group A and B:

	Group A	Group B		
	Mean ±SD	**Mean ±SD**	**MD**	**p value**
IgA (mg/dl)				
Pre treatment	426.37 ± 24.15	424.16 ± 17.69	2.21	0.65
Post treatment	168.13 ± 25.03	320.18 ± 27.76	-152.05	0.001
MD	258.24	103.98		
% of change	60.57	24.51		
	p = 0.001	***p = 0.001***		
IgM (mg/dl)				
Pre treatment	249.29 ± 15.29	247.68 ± 16.84	1.61	0.66
Post treatment	114.82 ± 16.20	190.76 ± 15.04	-75.94	0.001
MD	134.47	56.92		
% of change	53.94	22.98		
	p = 0.001	***p = 0.001***		
IgG (mg/dl)				
Pre treatment	1816.42 ± 110.50	1810.79 ± 105.94	5.63	0.82
Post treatment	904.11 ± 252.24	1567.73 ± 267.45	-663.62	0.001
MD	912.31	243.06		
% of change	50.23	13.42		
	p = 0.001	***p = 0.001***		

SD, Standard deviation; MD, Mean difference; p value, Probability value.

Discussion

This study was carried-out to investigate the influence of cupping therapy on immune system by detecting the levels of (T-cells, IL-6, IgA, IgM, and IgG) pre and post treatment in patients suffering from post-covid-19. The findings revealed that there were statistically significant differences between both groups in T-cells, IL-6, IgA, IgM, and IgG after 8weeks of treatment, with more favor to the study group.

The beneficial impacts of CT on various pains and disorders have been documented in numerous studies. CT, which has its roots in traditional medicine, is experiencing an increase in popularity, particularly in the Middle East, but also

beyond. More studies are required to fully understand the effectiveness of CT, but some have indicated its efficacy in certain disorders. One of the reasons CT is so common is that it doesn't include any chemicals or pharmaceuticals, and it hasn't been found to have any negative side effects when used in conjunction with other medications **(Mahmoud et al., 2013).**

According to a study conducted by Dons'koi et al. (2016), DCT reduces the quantity as well as activity of natural killer cells (NKc). According to a recent evaluation of cupping therapy, it has the potential to influence the immune system by boosting levels of immunological products like interferons, stimulating the complement system, and producing local inflammation **(Al-Bedah et al., 2018).**

The results revealed that of T-cells pretreatment of group A was 635.53 75± 148.13 ng/dl and that post treatment was 1030 ± 121.43 ng/dl. and the percent of change was 62.07%. Group A experienced a significant rise in T-cells after treatment compared to before treatment, with a mean difference of -394.47 ng/dl.

The results of this study come in agreement with a study of **Abbasi and Najafi, (2023)** who came to the conclusion that cupping therapy might be viewed as an adjunct treatment approach to influence the host microenvironment through the reduction of inflammation and the immune system through the enhancement of T-cell quality.

The improvement in this study may be attributed as External factors, like negative pressure and cuts, as well as internal factors, like modifications to pH, blood circulation, oxygen, released cytokines along with neurotransmitters, and the functioning of immune cells, specifically mast cell activation level, all contribute to the altered microenvironment of the triggered area in this neuroendocrine-immunomodulatory process. This is corroborated by the findings of Alipour et al. (2022), who found that patients with COVID-19 may experience immunomodulatory as well as anti-inflammatory benefits from cupping therapy.

The present study found that after treatment, there had been a mean difference of 215.21 ng/dl in T-cells across the groups. Patients in group A showed more improvement than those in group B, as indicated by a statistically significant rise in T-cells after treatment compared to group B.

Although antibody responses generated by a primary COVID-19 infection eventually fade, they may persist in the circulatory system for up to a year after symptoms first appear (PSO). Researchers have found that moderate disease is linked to robust antigen-specific T cells within 2 months post-immunization (PSO), but severe disease is linked to a lack of or an interruption in the production of these antiviral cells. **(Rydyznski et al., 2020 and Zhou et al., 2020).**

Since dry cupping as well as wet cupping both cause the skin to become red when applied, we decided to center our study on this inflammatory process. An injury's first line of defense is inflammation. Macrophages, neutrophils, dendritic cells, and NK (Natural Kill) cells are the main effector cells in this initial defense response. This is in line with the findings of Subadi et al. (2014), who found that the entrance of leucocytes into the injury marks the beginning of the cell reaction to the inflammatory process. After cupping therapy, the wounded area will likely have a preponderance of neutrophils and monocytes during the early stages of inflammation.

The findings of the present study revealed that IL-6 pretreatment of group A was 99.34 ± 22.61 pg/ml and that post treatment was 25.34 ± 4.92 pg/ml. There was a statistically significant reduction in IL-6 in group A following therapy compared to pretreatment levels; the mean difference among the two was 74 pg/ml, and the percentage of change was 74.49%.

Cupping raises the the generation of anti-inflammatory lipids (such as prostaglandin E1) and reduces the generation of pro-inflammatory lipids (including 12-HETE as well as Thromboxane B2) and inflammatory cytokines (e.g., IL-4, IL-5, IL-6, and TNF-α), according to a study by Dons'koi et al. (2016).

Hussam et al. (2015) conducted a study. It was shown that cupping treatment can fix blood chemistry problems caused by autoimmune diseases by filtering blood through arteries and veins in a way that depends on pressure and size. Reduced levels of autoantibodies, inflammatory mediators like IL-6, and serum ferritin (an important factor in autoimmunity) are the mechanisms by which cupping therapy inhibits immunopathogenesis.

Our study's findings are at disagreement with those of the latest review that suggested cupping could have an impact on the immune system by causing local inflammation, triggering the complement system, and raising levels of immune products like interferons, IL-6, IL-17, as well as IL-22 (Al-Bedah et al., 2018). This difference could be because cupping has bidirectional impacts on human immunoglobulins, which means it may improve irregular levels while having no apparent impact on normal levels. The reviewers also noted that the regulation outcome is linked to the initial function state.

Additionally, after treatment, there was also a mean difference of -39.27 pg/ml in IL-6 levels across the groups, as shown in the present study. After treatment, IL-6 levels in group A dropped significantly more than in group B, indicating that group A had a marked improvement over group B.

Zhanga and Li (2019) developed a cupping mice model and measured fatty acid levels in the skin and plasma of naked mice both before and after cupping treatment, and their results are in agreement with ours. The researchers looked at how cupping therapy affected the metabololipidome of polyunsaturated fatty acids (PUFAs). Results demonstrated that cupping treatment increased skin and plasma levels of anti-inflammatory lipids while decreasing levels of pro-inflammatory lipids. The results of this study demonstrated that cupping therapy, in contrast to anti-inflammatory drugs, decreases the secretion of IL-6 and TNF-α in vivo as well as that cupping treatment regulates the metabolic equilibrium between pro- and anti-inflammatory PUFAs.

The findings of this study contradict those of Mohammad Reza et al. (2012), who found no change in the amounts of IFN-g and IL-6 in venous blood two weeks following cupping. The reduplication of the cupping immunological response appears to be impacted, leading to a rise in IFN-g and IL-6 concentrations.

Group A's IgA value was 426.37 ± 24.15 mg/dl before treatment and 168.13 ± 25.03 mg/dl after treatment, according to the results of the present study. With a percentage change of 60.57 percent, the mean change from baseline to treatment was 258.24 mg/dl. Comparing to before treatment, group A's IgA levels dropped significantly after treatment. On the other hand, group A's IgM levels dropped from 249.29 ± 15.29 mg/dl to 114.82 ± 16.20 mg/dl. The average change from baseline to therapy was 134.47 mg/dl, or 53.94 percent. Group A's IgM levels dropped significantly after therapy compared to before. Additionally, group A's IgG value was 1816.42 ± 110.50 mg/dl before treatment and 904.11 ± 252.24 mg/dl after treatment. The average change from baseline to therapy was 912.31 mg/dl, or 50.23 percent. Group A's IgG levels dropped significantly after therapy compared to before.

Contrary to what Ranaei et al. (2004) found, this study The blood was examined for several markers, included IgA, IgG, IgM, Alb, TSH, T3, T4, Ca, CREAT, SGPT, SGOT, LDL, HDL, MCV, Hb, as well as HCT. Cholesterol, HDL,

LDL, along with FBS were the only parameters that showed statistically significant alterations. Interestingly our results showed that cupping therapy lower the number of IgA, IgM, IgG in blood. These results might be as cause of in **Ranaei et al., (2004)** post assessment were two weeks following cupping therapy which didn't give any difference in of IgA, IgM, IgG.

It was come in the agreement of **El- Dmyati et al., (2013**) researchers observed that cupping reduced levels of blood immunoglobins and increased levels of serum C3, two indicators of immune system abnormalities. The immune system may be impacted by cupping in three different ways. A primary effect of cupping is to stimulate the immune system by creating a false localized inflammation. Secondly, the complimentary system is activated by cupping. Thirdly, according to Shaban (2009), cupping raises levels of immune products like tumor necrotizing factor and interferon.

After treatment, the average post-treatment IgA level was -152.05 mg/dl. The levels of IgA in group A were significantly lower than those in group B after treatment. After treatment, there was a mean decrease of 75.94 mg/dl in IgM levels between the groups. After therapy, group A's IgM levels dropped significantly more than group B's, and eventually, there was a mean post-treatment IgG difference of -663.62 mg/dl across the groups. The levels of IgG in group A were significantly lower than those in group B after treatment.

Kang et al., (2022): concluded that the immune response post covid-19 returns its normal level even by cupping and pharmacological treatment or without any interference by about 1year post covid by normal body immune system.

Cupping could perform a protective function by enhancing immunity and so protecting the body against diseases, according to Khalil and colleagues (2013), who also suggested that cupping modulates the cellular component of the immune system and activates the complement system.

Cupping treatment, like anti-inflammatory medications, may be considered an adjuvant treatment approach to alter the host milieu through immune system modulation and inflammation reduction. Cupping has a two-way impact on human immunoglobulins; it normalizes abnormal levels, has no effect on normal levels, and regulates according to the initial function state (Abdullah et al., 2019).

It is clear that more study is needed to fully comprehend the immunological impact of cupping therapy, how it regulates hemoglobin and immunoglobulins, and how these effects vary across diseases and their severity.

Findings

The analysis of data showed that there was a significant improvement in T-cells, IL-6, IgA, IgM and IgG in the study and control groups with favor to the study group.

Conclusion

Dry cupping has a positive effect on the immune system, and inflammatory markers in patents with COVID-19. As an alternate therapeutic option for patients with problems with inflammatory proteins as well as hematological parameters, cupping therapy has a positive impact on older people.

Implementations

Dry cupping should be a part of Physical therapy rehabilitation of the immune system on post COVID-19.

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