بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

The Effect of Wet Blood Cupping on C Reactive Protein and Creatine kinase Levels

(A Study in Khartoum State)

تأثير الحجامة الدموية الرطبة على مستويات البروتين التفاعلي سي وإنزيم كيناز الكرياتين

(دراسة بولاية الخرطوم)

A dissertation submitted in partial fulfillment for the requirement of M.Sc. degree in medical laboratory science -Clinical Chemistry

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قال تعالى:

﴿ أَقِمِ الصَّلَاةَ لِدُلُوكِ الشَّمْسِ إِلَى غَسَقِ اللَّيْلِ وَقُرْآنَ الْفَجْرِ إِنَّ قُرْآنَ الْفَجْرِ كَانَ مَشْهُوداً ﴾

صدق الله العظيم

سورة الإسراء (الآية 78)

Dedication

With love and gratification I dedicate this work

To:

That person who hold my hands to teach me how I step up on my life; my mother

Person who protect and help me all the time, tolerate the Sun burn and difficulties for us

My father

Person who stand with me and continuing to support me my husband

My son I love you so much, you are the reason of my happiness, keep twinkling on my sky, god bless you

My sisters, brothers, friends, and relatives for their support

To all UN visible hand workers

To my country

Acknowledgments

First great thanks referring to Allah for his mercies and guidance to live and achieve my goals.

Secondly it gives me a pleasure and most honored to become supervised by such nice person **Dr. Abd eLgadir ELmogadum** ; whom didn't keep effort to help me and accept my annoyance with big heart and nice smile .

I really appreciating your kind cooperating, I hardly express my gratitude.

Also special thanks to all members of Sudan university for science and technology (SUST); specially to staff members of clinical chemistry..

Great thankfulness to volunteers for their nice dealing with the research demands without any growl; as same as cupping centers staff . Specially Dr. Mona Jafar .

Not forget to thank underhanded people for their help, and support.

Abstract

Hejamah refers to wet cupping in Muslims and Arab cultures. It being used for decades and advised by Prophet Mohammed peace be upon him. This study was carried out to evaluate the effect of wet cupping on CRP and CK levels; both have vital role and diagnostic value. The study was carried on forty volunteers at Khartoum cupping centers from April to August (2017), venous blood samples was collected, processed, and analyzed before and after by using of BTs-350 semi-automated chemical analyzer; quantitative methodology was done for the CRP measurement. The obtained results were analyzed statistically by using of SPSS software program.

The level of CRP was significantly decreased with p value 0.01.in post cupping compared to pre cupping samples of the same volunteers (mean ±SD:29.48 ±44.97 versus 17.88±27.71 mg/L) While the level of CK was insignificantly different with p value 0.45 in post cupping compared to pre cupping samples of the same volunteers (mean ±SD:80.35±56.38 versus 87.70 ±56.7 U/L).The blood pressure also measured and was significantly decreased with p 0.00 in post cupping compared to pre cupping samples of the same volunteers (mean ±SD:103.06 ±13.13versus 98.03±11.74 mmHg). The results showed no correlation between the CK and CRP in pre and post cupping (r= -0.239, p value 0.155/ r= -0.223, p value 0.184) respectively.

In conclusion: blood cupping affects the CRP, BP by decreasing them, and increasing CK level.

المستخلص

الحجامة تعني الامتصاص بواسطة كوب اوأي أداة أخرى ولها عدة أنواع منهـا الجافـة والرطبة وهي التي تمارس عند العرب والمسلمين منذ القدم وقد وصى الرسول صلى الله عليـه وسلم بممارستها في كثير من الاحاديث حتى انه احتجم عدة مرات. تمت هذه الدراسة لتحديد تأثير الحجامة الرطبة علي البروتين المتفاعل(سي) أو البروتين الإرتكاسي وأيضا على إنزيم كينـاز الكرياتين : حيث لكل منهما قيمة حيوية وتشخيصية عالية .أجريت الدراسة على اربعين متطوعا بمراكز حجامة بولاية الخرطوم في الفترة الزمنية من فبراير إلى يونيو (2017). حيث تم جمـع عينات الدم قبل وبعد الحجامة ومعالجتها بالطرق المثالية ومن ثـم تحليلهـا بإسـتخدام جهـاز بايوسستم 350 للتحليل الكيميائي وذلك بواسطة طرق كمية لتحديد تركيز البروتين التفاعلي سـي وإنزيم كيناز الكرباتين قبل وبعد الحجامة لتتم المقارنة بينهما إحصـائيا عـن طريـق اسـتخدام البرنامج الإحصائي المحوسب (اس بي اس اس) الحزمة الإحصائية للعلوم الإجتماعية.

أظهرت الدراسة ان متوسط مستوى المتفاعل الإرتكاسي إنخفض بصورة ملحوظـة بعـد الحجامة مقارنة مع قبلها لنفس المتطوعين (قبل: 29.48) و(بعد 17.88) مع احتمال إحصائي (0.01).

أظهرت الدراسة ان متوسط مستوى إنزيم كيناز الكرياتين زاد بصورة ملحوظة بعد الحجامـة مقارنة مع قبلها لنفس المتطوعين (قبل: 80.35)و(بعد 87.70) مع احتمال إحصائي (0.45).

أظهرت الدراسة أنه لا توجد أي علاقة إرتباط بين انزيم كيناز الكرياتين والبروتين الإرتكاسـي قبل وبعد الحجامة بناء على أن معامـل بيرسـون (-0.223و-0.239مـع معنويـة 0.155 و0.184) على التوالي.

ما يستفاد من الدراسة أن الحجامة تؤثرعلى مستوى البروتين التفاعلي سـي وكينـاز الكريـاتين وكذلك ضغط الدم .

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List of abbreviations

ADP	Adenosine di phosphate
AMI	Acute myocardial infarction
AS	Alyhe alsalam
ATP	Adenosine tri phosphate
BMI	Body mass index
BP	Blood pressure
C/EBPβ C/EBPδ	Enhancer binding protein ;beta&delta
CK	Creatine kinase
CNS	Central nervous system
CPS	Causative pathological substances
CRP	C reactive protein
CVD	Coronary vascular diseases
DVT	Deep vein thrombosis
ESR	Erythrocytes sedimentation rate
HTN	Hypertension
IL-6	Inter leukin 6
Iq23.2	CRP gene located on chromosome one
LDL	Low density lipoprotein
MCRP	Monomeric C reactive protein
PCRP	Pentameric C reactive protein
RA	Radi allah anho
SAW	Sala allah alyhe wasalm
SD	Standard deviation
TNF	Tumor necrosis factors

Chapter one

Introduction

Chapter one

Introduction, rationale, objectives

1.1 Introduction:

Cupping is a well-known traditional healing remedy in many parts of the world. Wet cupping is defined as “the process of using a vacuum at different points on the body but with incisions in order to remove ‘harmful’ blood which lies just beneath the surface of the skin” (Mahdavi et al., 2011). It is known in Arabic language as hijama. For Muslims, it has special importance because it was recommended by the Prophet Mohammed (peace be upon him) on many occasions. For example, he said, “Indeed the best of remedies you have is hijama (cupping)” ” (Mahdavi et al., 2011). Cupping is thought to remove noxious materials from skin microcirculation and interstitial compartment (Goodwin & McIvor., 2011). Wet cupping has been claimed to drain excess fluids and toxins loosen adhesions and lift connective tissue, bring blood flow to skin and muscles, and to stimulate the peripheral nervous system (Lee et al., 2011). Also, cupping is reducing pain and high blood pressure as well as modulates neurohormones and the immune system, cupping therapy is also used to improve subcutaneous blood flow and to stimulate the autonomic nervous system (Yoo & Tausk., 2004). There are three types of cupping; dry, wet, and massage cupping. In dry cupping stationary cups are placed on the skin and left for a period of five to 15 minutes in one location without incisions, while in wet cupping (hijama) the process of using a vacuum at different points on the body but with incisions in order to remove 'harmful' blood which lies just beneath the surface of the skin. In massage cupping, oil is applied on the skin to facilitate smooth movement and discover the areas of tension and congestion prior to applying

the cup. Cupping can be used on the neck, shoulders, back, sacral area, hip, abdomen, thigh, upper arms and calves. Cupping (hijama) is an alternative medicine that can treat many diseases ranging from minor to serious chronic diseases such as arthritis and diabetes(Cao et al., 2011).

C Reactive Protein(CRP): is a sensitive acute-phase protein, an important but nonspecific aspect of the immune response. That is, CRP appears in the blood following infection or tissue damage. Its name is derived from the discovery that it binds to the cell wall of the C-polysaccharide of Streptococcus organisms, which helps phagocytic cells to destroy these and other pathogens. It also binds to tissue breakdown products released following myocardial infarction, stress, surgery, trauma, and infection and aids in resolution of inflammatory responses (WHO, 2014)**.** Creatine kinase (CK); is a member of the phosphagen kinase family and catalyzes the reversible phosphotransfer between the ATP/ADP and Creatine/Phosphocreatine systems. CK is highly expressed in excitable tissues that require large energy fluxes and plays a significant role in the energy homeostasis of these tissue cells(Teixeira and Borges., 2012).

There was no study which explain the correlation of CK and CRP before and after cupping ,but there were some studies carried on CRP such as farahmand etal (2014) Study; which found no effect of wet cupping on hs-CRP, and Hussam et al(2015) study; found that wet cupping effect on CRP. Another study of Mohammad Khalil et al(2016) which carried on CK showed that wet cupping affect the CK blood level.

1.2 Rationale: Recently, Loukas et al reported that medical knowledge gained from Qur'an (spoken word of God) and hadeeths (spoken word of prophet Muhammad peace be upon him) can be an important source of humanity medicine in light of their astonishing agreement with modern

medical knowledge (Loukas et al., 2010). also there is revolution of alternative medicine, hence there is a lack of studies which explain the exact effect of blood cupping on CK&CRP blood level ;both of them considering as markers for many conditions. In addition to Sudanese people practicing wet cupping from decades with lack of scientific studies which explain the effect.

1.3 Objectives:

1.3.1 General Objective:

To assess the effect of wet blood cupping on CRP & CK blood level.

1.3.2 Specific Objectives:

1. To measure plasma CRP and CK levels pre and post cupping.
2. To measure blood pressure pre and post cupping.
3. To correlate between CRP and CK before cupping .
4. To correlate between CRP and CK after cupping.
5. To correlate between BMI and age.

Chapter two

Literature review

Chapter two

Literature reviews

2.1 Blood cupping:

2.1.1 Definitions:

Blood cupping is an operation of drawing some blood by applying a cup to scarified skin **(**Majid et al.,2007) where a cup is attached to the specific area of skin surface along the back to cause local congestion through the negative pressure created **(**Ranase et al.,2004) In that practice, the skin is pinched, sometimes at specific point (e.g. bladder meridian points), generally the areas of the body that are fleshy, are preferred sites for cupping, the cup is left for about five minutes until a redness is generated **(**Wang,1996). Blood cupping has the function of warming and promoting the flow of energy in the blood thus dispelling cold, dampness, toxins, and winds and as well as diminish pain (Ju, 1998) The Arabic name for cupping therapy is Al-Hejamah which has been part of middle eastern cultural practice for thousands of years with citations dating back to the time of Hippocrates (400BC) (Curtis, 2005). Wet cupping is defined as "the process of using a vacuum at different points on the body but with incisions in order to remove 'harmful' blood which lies just beneath the surface of the skin" (Mahdavi et al., 2011). Hejamah is a traditional Islamic treatment and a part of the prophetic medicine recommended by the prophet Mohammed, Peace Be Upon Him (PBUH) , and it is used for the treatment of a variety of medical conditions (El Sayed et al**,**2013). Hejamah is a form of cupping therapy, also known as wet-cupping, blood leach or blood-letting therapy (El Sayed et al**,**2013.,Cao ,2010). According to the prophetic medicine, hejamah should be performed at specific times during the odd days of full moon (day

17, 19 and 21) in the lunar months (Islamic calendar) and the patients should be fasting (Mahmoud, 2013).

2.1.2 Historical back ground:

It is recorded in the books of Ahadeeth that amongst other things, such as the use of the turban and miswak, hijamah was a practice of every Nabi (AS). Considering that the Quran clearly states that every nation was sent a guide, and the fact that at least 124 000 Ambiyaa (AS) were sent to this world, Hijamah as a treatment is to be found throughout the world as a result of this long history of continuous use. Indeed historical texts prove that this is the case with depictions of cupping equipment being seen on ancient stone tablets and markings from archeological findings throughout the world (Feroz, 2013).

The earliest historical evidence of the use of Hijamah is from the ancient Egyptians. One of the oldest Egyptian medical textbooks, written in approximately 1550 BC, describes "bleeding" used to 'remove pathogens from the body'. It is evident that bloodletting was considered a remedy for almost every type of disease as well as an important means of preserving good health and life (Feroz, 2013).

Hippocrates and Galen were also great advocates of Hijamah. In Hippocrates' time bloodletting was topological and not used in terms of the theory of the 4 humors. Specific points were bled for specific illnesses. Galen explains that the principle indication for bloodletting is to eliminate residues or divert blood from one part of the body to another.

His approach was based on two key Unani concepts prevalent at the time. First, that blood did not circulate well in the body, and that it eventually went stagnant until it was "let out". Secondly, the concept of the balance of the four humors (blood, phlegm, black bile and yellow bile) was the source

of health or illness, in which case bloodletting is used to bring about balance between these humors. Mapping out the blood vessels of the body, Galen would cut his patients in different areas; depending on what area he wanted to treat.

In the Middle East region we find that the practice of Hijamah was already present before the arrival of the final Rasul (SAW) and the final Nabi (SAW) both encouraged and used it himself on many occasions.

Ibn Sina, the famous Muslim physician said: 'Hijamah is not preferred in the beginning or the end of the month. It is preferred in the middle of the month when the substances (of the constitution or the condition) accumulate and become agitated.

The Talmud included rules for days where bloodletting could be practiced and early Christian writings also outlined which days were the best for bloodletting therapy (Feroz, 2013).

In the East, Bloodletting and wet cupping was always an integral part of the medical practices, and remains so to this day. The ancient Chinese medical text which is widely regarded as the oldest medical text in existence, the Nei Jing, or Inner Classic says that: "if there is stagnation it must be first be resolved through bloodletting before the application of acupuncture or moxibustion.". Another ancient Chinese medical text the Su Wen gives detailed instructions for piercing combined with bloodletting but forbidding the letting of blood in certain seasons. The Su Wen states: "When heaven is warm and when the sun is bright, then the blood in man is rich in liquid and the protective qi (energy/lifeforce) is at the surface Hence the blood can be drained easily, and the qican be made to move on easily…". Some researchers believe that acupuncture actually began as bloodletting, with sharp objects being used to bleed the acupuncture points before the

widespread use of needles to perform acupuncture. This is also evidenced by depictions of ancient “needles” which were more akin to bleeding instruments than the fine acupuncture needles in use today. The Lingshu (Spiritual Pivot) and its companion volume, the Su wen (Simple Questions) , written around 100 B.C., established the fundamentals of traditional Chinese medical ideas and acupuncture therapy. Originally, there was a set of 9 acupuncture needles, which included the triangular lance, sword like flat needles, and fairly large needles. Regarding the fourth needle, which has a tubular body and lance-like tip, the text states: “This can be used to drain fevers, to draw blood, and to exhaust chronic diseases?" The seventh needle is described as being hair fine (corresponding to modern acupuncture needles); it is said to "control fever and chills and painful rheumatism in the luo channels." In modern practice, using the lance as a means to treat chronic diseases has been marginalized (except to treat acute flare-ups of chronic ailments), while the applications of the hair-fine needle has been greatly expanded beyond malarial fevers and muscle and joint pain. Traditional Chinese Medicine and Acupuncture practitioners still use bleeding therapies though it is more commonly practiced in China than by western practitioners due to concerns about infection and the general dislike for dealing with blood in the acupuncture clinic.

North American natives are reported to have used buffalo horns for wet cupping. The horns were hollowed with a small hole at the top through which the cupper would suck the air out of, in order to create a vacuum in the horn which would then pull up the blood from the incisions previously made with a blade. Buffalo horns are also reported as being used for hijamah during the Babylon - Assyrian Empire (stretching from Iraq to the Mediterranean). Bloodletting became widespread during the middle ages and

surprisingly, became a practice common to barbers who would display a “bloodletting pole” outside their establishment to indicate that they practiced bloodletting (Feroz, 2013).

2.1.3 Reasons for having Hijamah:

Besides the general effects of Hijamah in improving and maintaining good health, especially in the hot regions, the Nabi (SAW) also used and recommended Hijamah for specific illnesses.

2.1.3.1 Injury:

Jaabir ibn Abdullaah (RA) reported that the Rasul (SAW) fell from his horse onto the trunk of a palm tree and dislocated his foot . Waki ' (RA) said, "Meaning the Rasul (SAW) was cupped on(his foot) for bruising." (Saheeh Sunan ibn Maajah (2807)).

2.1.3.2 Headaches:

Salma (RA), the servant of the Rasul (SAW) said, "Whenever someone would complain of a headache to the Rasul of Allaah (SAW), he (SAW) would advise them to perform Hijamah." (Saheeh Sunan abi Dawud (3858)).

2.1.3.3 Sihr (black magic):

Ibn al-Qaiyum (RA) mentions that the Rasul (SAW) was cupped on his head when he was afflicted with sihr and that it is from the best of cures for this if performed correctly. (Zaad al Ma'aad (4/125-126)).

2.1.3.4 Poison:

Abdullaah ibn Abbas (RA) reported that a Jewish woman gave poisoned meat to the Rasul (SAW) so he (SAW) sent her a message saying, "What caused you to do that?" She replied, "If you really are a Nabi then Allaah will inform you of it and if you are not then I would save the people from you!" When the Rasul (SAW) felt pain from it, he (SAW) performed

Hijamah. Once he travelled while in Ihram and felt that pain and henceperformed hijamah. (Ahmed (1/305) the Hadeeth is Hasan).

2.1.4 Contra-indications and precautions to treatment:

Cupping Therapy has no major side effects aside from minimal discomfort due to the method of application of skin cuts to the patient. In cases where the patient's pain threshold is low, a local anesthetic can be administered. Also other possible minor side effects that may occur is the feeling of slight light headedness post Cupping Therapy, this again is similar to the sensation one feels after having had blood taken from the doctor, as Cupping Therapy encourages blood flow to the cupped region (hyperemia), one may therefore feel warmer and hotter as a result of vasodilatation taking place and slight sweating may occur. Again this can be attributed to sound scientific rationale and there is no cause for concern. Pregnant women or menstruating women, cancer (metastatic) patients and patients with bone fractures or muscle spasms are also believed to be contra-indicated. Also, Cupping Therapy cannot be applied to a site of DVT, where there are ulcers, arteries or places where a pulse can be felt (Chirali, 1999).

2.1.5 Modern understanding of cupping therapy:

In light of modern medicine and prophetic medicine concludes that cupping therapy does not work through establishing the balance between positive and negative or Yin and Yang, which is against scientific thinking and has no medical background. Instead, Taibah theory for scientific mechanisms of cupping therapy is recently published as a novel evidence-based mechanism for explaining scientific and medical bases of cupping therapy (Mahdavi et al., 2011). Taibah theory explains on scientific bases how cupping therapy works through clearing blood plasma and interstitial fluids from CPS. Negative pressure introduced through sucking cups creates

skin uplifting inside which interstitial fluids, filtered fluids from blood capillaries, hemolyzed blood cells (but no intact blood cells) and CPS are Based on Taibah theory, indications for Al-hijamah include treating diseases that will benefit maximally or partially from clearing blood and interstitial spaces from CPS through excreting excess intravascular fluids, excess interstitial tissue fluids and excretion of other CPS while taking into account the other health-based benefits of Al hijamah. An example for increased extracellular and interstitial fluid volume is hypertension (Zhang, 2010) that was reported to improve on WCT (Boncler and watala, 2009) as fluid overload was reported to play an important role in the pathogenesis and development of salt-dependent hypertension (Gavras and Gavras ., 2012). An example for toxic CPS in interstitial fluids is cellulitis. Cellulitis is a local form of suppurative inflammation of the skin and underlying tissues with an accompanying increase in inflammatory tissue fluids (exudate) together with increased CPS in the form of bacteria and bacterial toxins (CPS) (Van Amersfoort et al,2003). Cellulitis was reported to improve on using WCT (Mahdavi et al,2011., Ahmed et al,2011). An example for increased inflammatory CPS is chronic osteoarthritis. Chronic osteoarthritis is a chronic inflammatory process initiated by proinflammatory cytokines e.g. interleukin-1 and tumor necrosis factor-1 alpha together with an acceleration of cartilage degradation process through increased levels of matrix metalloproteinases, stromelysins, gelatinase and plasminogen activators (Hassan and Oyoo, 2011). All those CPS are catalyzing the pathogenesis of osteoarthritis. Al-hijamah-induced clearance of those CPS from blood plasma and interstitial fluids may explain on scientific bases the improvement reported by chronic osteoarthritis patients upon treatment with

Al-hijamah. .

2.1.6 Classification of cupping therapies:

Cupping therapy types can be classified into six main categories, the first is technical category which includes dry, wet, massage and flash cupping therapy. The second is the power of suction related category which includes light, medium, strong and pulsatile cupping therapy. The third is method of suction related category which includes fire, manual vacuum, and electrical vacuum cupping therapy. The fourth is based on materials inside cups, and includes herbal, water, laser, Moxa, needle, electrical stimulation, and magnetic cupping therapy. The fifth is area treated related category. The sixth is other cupping methods category that includes sports, cosmetic and aquatic cupping.

2.1.6.1 Dry cupping:

Dry cupping (Lauche, 2011)is also given other names such as static cupping or retained cupping (Cao et al., 2011). This method of applying cups over the skin needs negative pressure inside the cups through various methods including fire, manual pump or electrical suction (Kim et al., 2011). Negative pressure is the pressure that is less than ambient pressure, and created by exhausting air inside the cup. The practitioners leave the cups on the skin area up to 15 minutes. The pressure inside the cup can be controlled by the number of suctions when using manual pump. Increasing number of suctions will increase the negative pressure inside the cup (Tham et al., 2006).The pressure inside the cup can also be controlled by the fire exposure time when using fire to create negative pressure. Prolonged exposure of the cup to the fire will increase the negative pressure inside the cup that may cause pain or discomfort and may cause skin burn due to the overheating of the cup. Atmospheric (ambient) pressure is higher than the negative pressure

inside the cup allowing the skin to pullout. Cupping is applied to increase the circulation of blood and lymph to the local area and also to relieve painful muscle tension (Lauche, 2011). Cupping effectively treats pain and also enhances a patient's general feeling of wellbeing (Lauche , 2011) Risk of burn, scar formation, and dermatitis are the main disadvantages of this method.

2.1.6.2 Flash cupping:

Flash cupping(Cao et al., 2012), also referred to as empty cupping (Cao et al., 2010)is the name given when several medium to light pressure cupping are preformed several times in quick succession along the area being considered for treatment that requires stimulation(Cao et al., 2010) . It only takes less than 30 seconds from the time when cup is applied and then removed because it entails stimulation process. It is done by using one cup, or some practitioners use four medium sized cups. They apply the four cups quickly then reapply them on the skin of next area before 30 seconds and used to stimulate para spinal lines on the back. This method is used when dry cupping is not indicated especially in young people and ladies(Cao et al., 2010) .

2.1.6.3 Wet cupping:

Wet cupping ha(Kim et al., 2011)s been given several other names: full cupping(Al-Rubaye, 2012),Bloodletting cupping (Ahmed et al ., 2004)and bleeding cupping (Chen and Zheng , 2010).This method is used most frequently in traditional medicine (Cao et al., 2010). A surgical instrument is used to scrape the skin and the cup is then applied to suck blood (Ahmed et al ., 2004).Laceration of the skin and capillary vessels takes place in wet cupping, and it may act as a nociceptive stimulus, that triggers diffuse noxious inhibitory control (DNIC). It may help in treatment of chronic

musculoskeletal pain (Kim et al., 2011).Skin disinfection, wearing personal protective equipment, following infection control program that includes safe medical waste disposal are advisable for all cupping therapy practitioners. The risk of infection, vasovagal attacks and scars are the main disadvantages of this method.

1.2.6.4 Massage cupping:

Massage cupping (Jiang et al ., 2004)also known as moving cupping(Cao et al., 2010),dynamic cupping (Winkes et al., 2012)and gliding cupping (Tham et al., 2006) is a method of massage and done by applying oil to the skin and moving the cup, by a weak suction, on the area that needs massage (Tham et al., 2006).Various types of oils may be used such as olive oil, peppermint oil and lavender oil. It is suitable for all people, even young and elderly people. The cost may be the disadvantages of this method.

Two distinct wet cupping methodologies were reported in the literature. Both methods are in agreement with each other as regard the starting steps, which include demarcation of skin points to which cupping therapy should be applied, followed by sterilization at these sites and as regard the last step, which is sterilization. They differ in the order and number of the steps of cupping treatment itself i.e. what comes first: cupping step (negative pressure suctioning) or puncturing skin step. The difference in the two methods may reflect different historical origins in both methods of cupping therapy based on the known standard protocols in their countries of origin . in Chinese clinics in which the five steps of wet cupping therapy are: demarcation (marking cupping points by pen), sterilization, puncturing (lanceting skin in 2 mm-depth), cupping (with manual pump suction for few minutes) and sterilization (Cao et al., 2010). This method is better to be abbreviated to PC (puncturing and cupping) method. In another Chinese

report for treating 140 cases of fibrositis (Zhang , 2009) and another Chinese study for treating gouty arthritis using wet cupping therapy, the authors reported the same steps of the PC method (Zhang, 2010). In a German study for treating carpal tunnel syndrome with wet cupping therapy, authors mentioned same steps reported by the previous Chinese studies (Michalsen et al., 2009), which give the impression that German physicians got their cupping method from Chinese medicine. Other European peoples use mainly the PC method of wet cupping therapy (Salomonsen, 2011). In the Arab literature, a different six-step method of wet cupping therapy is reported in prophetic medicine which includes an extra step (cupping step before skin puncturing) i.e. steps ofthe Arabic methodology for wet cupping therapy (Al-hijamah) are skin demarcation, sterilization, cupping, puncturing, cupping and sterilization. This method is better to be abbreviated to CPC (cupping, puncturing and cupping) method. Interestingly, a Chinese report described steps of the currently practiced wet cupping therapy method in Saudi Arabia (Hany, 2013), which is exactly the CPC method as reviewed early in prophetic medicine (Mohamed, 2005). In cupping therapy, a cupping glass is applied to a predefined skin area, and a vacuum is generated by mechanical withdrawal or thermal cooling of the entrapped air under the cup. The skin is then dragged into the cupping glass, resulting in rubor and heat at the affected area with increased perfusion (Tue et al., 2012). Also, flaming is used for suction (minus pressure) inside the cups, so as to apply it promptly on the desired part of the body. Cupping is thought to act mainly by increasing local blood circulation and relieving the painful muscle, it mainly involves improving microcirculation, promoting tension. Operation is performed in cupping inter scapular region, at the right and left carotid, at the lateral side of the neck, at protuberance behind the ear, the middle and

crown of the head, at the chin, at the thighs or folds of the thighs, at knee joints, at ankle joints, at the breast, at hips or buttocks, at the anal area, at wrist joints, at ear tragus, and at shoulder joints (Iqbal and Ansari, 2013). The back, chest, abdomen, and buttock, areas of abundant muscle are the most common sites on which the cups are applied (Yoo and Tausk, 2004).The cups are typically left in place for 5-10 minutes, or sometimes more. Owing to vasodilatation and edema, histological changes are observed in the skin without any cellular infiltrate. The after effects of cupping often include erythema, edema, and ecchymosis in a characteristic circular arrangement. Most of the local skin changes subside within a few weeks (Al-Rubaye, 2012).

2.2 C-reactive protein(CRP):

Is an acute-phase protein that serves as an early marker of inflammation or infection? .The protein is synthesized in the liver and is normally found at concentrations of less than 10 mg/L in the blood. During infectious or inflammatory disease states, CRP levels rise rapidly within the first 6 to 8 hours and peak at levels of up to 350–400 mg/L after 48 hours (WHO, 2014). CRP binds to phosphocholine expressed on the surface of damaged cells, as well as to polysaccharides and peptosaccharides present on bacteria, parasites and fungi (WHO, 2014). This binding activates the classical complement cascade of the immune system and modulates the activity of phagocytic cells, supporting the role of CRP in the opsonization (i.e. the process by which a pathogen is marked for ingestion and destruction by a phagocyte) of infectious agents and dead or dying cells (WHO, 2014). When the inflammation or tissue destruction is resolved, CRP levels fall, making it a useful marker for monitoring disease activity (WHO, 2014).

2.2.1 C-Reactive Protein Structure and Metabolism:

CRP was first described in 1930 by Tillet and Francis, named after its ability to precipitate and interact with phosphorylcholine residues of the C polysaccharide derived from teichoic acid within the cellular wall of Streptococcus pneumoniae, as well as its ability to precipitate with calcium ions (Ridker, 2009). Although CRP is classically considered an important regulator of the innate immune system and a paramount mediator of the acute-phase response (Ridker, 2009), it has also been associated with various chronic inflammatory processes, such as certain rheumatologic conditions, cancer, and CVD (Dhingra et al., 2007) . CRP is a 206-amino acid member of the short pentraxin family, alongside serum amyloid P component (SAP), with high phylogenetic conservation (Mantovani et al., 2008). Pentraxins share a characteristic structure: five identical nonglycosylated globular subunits—each of which is constituted by two β-pleated sheets—which are noncovalently associated and arranged in a symmetric cyclic pattern around a central pore, determining a pentameric, discoidal, and flattened configuration (Thompson et al., 1999). C-reactive protein is predominantly synthesized in the liver (1q23.2) (Heres et al., 2011**)** , typically within the transcriptional phase of the response to pro inflammatory cytokines. IL-6 appears to be the main regulator, by promoting de novo synthesis of CRP via upregulation of C/EBPβ and C/EBPδ, key transcription factors in this process (Black et al, 2004). In addition, IL- 6 signaling may be reinforced by IL-1β and TNF, both of which increase transcription rate of CRP (Nanri et al,2007) . Serum CRP levels have also been closely linked to signaling by proinflammatory cytokines released by visceral adipose tissue (Brooks et al., 2010). Indeed, both hypoadiponectinemia and hyperleptinemia, two adipokine disturbances common in subjects with obesity and insulin

resistance, have been linked to increased hepatic production of CRP (Scotece et al 2012. ,Puglisi and Fernandez,2008.) , as well as augmented *in situ* synthesis of CRP in vascular endothelial cells in hyperleptinemia (Singh et al., 2007). In this regard, adipose tissue has been well characterized over recent decades as an endocrine organ, with important immunomodulatory roles through release of inflammatory messengers such as IL-1β, IL-6, and resistin, among others (Kwon and Pessin,2013), underlying the chronic inflammatory component of obesity (Ouchi et al., 2011**)** . Adipocyte dysfunction is the central phenomenon in this scenario (Taube et al,2012) encompassing hyperplasia and hypertrophy of these cells due to lipid storage increase, which leads to hypoxia, rupture propensity, and ultimately proinflammatory adipokine secretion in this tissue (Monteiro and Azevedo, 2010).

Moreover, CRP has been shown to be expressed in adipocytes in response to proinflammatory mediators, representing yet another link between obesity and chronic inflammation (Calabro et al., 2005). Following synthesis and release into circulation, serum CRP levels tend to increase significantly 6–8 hours after initial stimulation, peaking at 24–48 hours, with a half-life of approximately 19 hours. CRP concentration in circulation is primarily determined by its synthesis rate (Trujillo and Scherer, 2006). Although the liver is the main site for production and release of CRP, its mRNA has been found in a myriad of extrahepatic sites, including adipose tissue, lungs, epithelial cells of renal cortical tubules, lymphocytes, and atherosclerotic lesions, in both macrophages and smooth muscle cells (Juan et al., 2014). Numerous studies have focused on identifying other extra hepatic sources of CRP production that may underlie the lower and more sustained CRP concentrations which appear to predict cardiovascular risk; these include

findings of CRP synthesis in coronary smooth muscle cells in response to inflammatory cytokines. Locally produced CRP may play an important role on endothelial cell activation (Calabro et al., 2003).

2.2.2 C-Reactive Protein: From Pentameric to Monomeric:

CRP has been described to adopt two different conformational forms: the native pentameric isoform (pCRP) and the monomeric isoform (mCRP) (Kobayashi et al., 2003) , which possess distinct antigenic, electrophoretic, and biological features (Huang et al., 2009) . Although pCRP is the main form detected in serum (Filep, 2009) and appears to be a very stable molecule, current evidence suggests that conformational subunits from pCRP can be dissociated, both in vitro and in vivo, into individual mCRP units. On the other hand, independent mCRP synthesis may also be an important source of this form (Boncler and Watała, 2009)

2.2.3The Role of C - reactive protein:

It may have a general function in defence against bacteria and foreign substances. Its concentration can increase 30-fold from a normal value of less than 5 mg/L during the acute phase response, for which it is a valuable marker, particularly in the context of monitoring patients with inflammatory conditions such as rheumatoid arthritis and Crohn’s disease. Its measurement appears to be both more sensitive and more specific than measurements of the It may have a general function in defence against bacteria and foreign substances. Its concentration can increase 30-fold from a normal value of less than 5 mg/L during the acute phase response, for which it is a valuable marker, particularly in the context of monitoring patients with inflammatory conditions such as rheumatoid arthritis and Crohn’s disease. Its measurement appears to be both more sensitive and more specific than measurements of the erythrocyte sedimentation rate

(ESR) and plasma viscosity in this respect. The CRP concentration begins to rise at about 6 h after the initiation of an acute phase response and reaches a peak after about 48 h before beginning to fall (William et al., 2012).

In the Pathophysiology of Atherosclerosis Inflammatory mechanisms play a fundamental role in all phases of atherosclerosis, from the initial recruitment of circulating leukocytes to the rupture of unstable plaques. Among multiple inflammatory biomarkers, CRP boasts the largest body of research supporting its role as an independent risk factor in the development of CVD, as it actively participates in atherogenesis by directly influencing processes such as activation of the complement system, apoptosis, vascular cell activation, monocyte recruitment, lipid accumulation, and thrombosis. Both isoforms are involved in such processes: pCRP can generate inflammatory responses binding to the phosphatidylcholine on the exterior of LDLox and the surface of apoptotic cells, while mCRP is able to modulate platelet function inducing aggregation and contributes to atherothrombotic complications by promoting thrombosis (Juan et al, 2014).

2.3Enzymes:

Enzymes are specific biological proteins that catalyze biochemical reactions without altering the equilibrium point of the reaction or being consumed or changed in composition. The other substances in the reaction are converted to products. The catalyzed reactions are frequently specific and essential to physiological functions, such as the hydration of carbon dioxide, nerve conduction, muscle contraction, nutrient degradation, and energy use. Found in all body tissue, enzymes frequently appear in the serum following cellular injury or, sometimes, in smaller amounts, from degraded cells. Enzymes catalyze many specific physiological reactions. These reactions are facilitated by the enzyme structure and several other factors. As a protein,

each enzyme contains a specific amino acid sequence (primary structure), with the resultant polypeptide chains twisting (secondary structure), which then folds (tertiary structure) and results in structural cavities. enzyme contains an Active site, often a water-free cavity, where the substance on which the enzyme acts (the substrate) interacts with particular charged amino acid residues. An allosteric site (a cavity other than the active site) may bind regulator molecules and, thereby, be significant to the basic enzyme structure (Michael et al.; 2010).

Enzymes can be classified on the basis of the type of reaction it catalyzes (International Union of Biochemists [IUB] classes). They are:

1. Oxidoreductases: Involved in oxidation and reduction of substrates.
2. Transferase: Help in transfer of a particular group such as methyl or glycosyl groups from one substrate to another.
3. Hydrolases: Bring about hydrolytic cleavage of bonds like C-C, C-O, C-N, etc.
4. Lyases: Facilitate removal of small molecule from a large substrate leaving double bonds; also add groups to double bonds.
5. Isomerases: Isomerisation of substrate.
6. Ligases: Involved in joining together of two substrates, coupled to the hydrolysis of an ATP (Michael et al.; 2010).

2.3.1 Creatine kinase:

CK is an enzyme with a molecular weight of approximately 82,000 that is generally associated with ATP regeneration in contractile or transport systems. Its predominant physiologic function occurs in muscle cells where it is involved in the storage of high-energy creatine phosphate. Every contraction cycle of muscle results in creatine phosphate use, with the

production of ATP. This results in relatively constant levels of muscle ATP. The reversible reaction catalyzed by CK is shown

In following Equation:

$$\text{creatine} + \text{ATP} \xrightarrow{\text{CK}} \text{creatinephpsphate} + \text{ADP}$$

2.3.2.1Tissue Source

CK is widely distributed in tissue, with highest activities found in skeletal muscle, heart muscle, and brain tissue. CK is present in much smaller quantities in other tissue sources, including the bladder, placenta, gastrointestinal tract, thyroid, uterus, kidney, lung, prostate, spleen, liver,

And pancreas (Michael et al.; 2010).

Creatine kinase has three isoenzymes, which:

- CK1 or CKMM, found mostly in skeletal muscle.
- CK2 or CKMB, found predominately in cardiac muscle.
- CK3 or CKBB, found in smooth muscle. (Nessar, 2011) .

2.3.1.2 Structure and functions:

The cytosolic CKs (MMCK and BBCK) are highly conserved in their sequence and share and almost superimposable terciary structure. They exist as a dimer in solution, each subunit composed of two domains: a smaller N-terminal domain containing only a-helices and a larger C-terminal domain with both b-sheets and a-helix secondary structures. The enzyme active site is located at the cleft of the two domains and is thought to facilitate the entry of substrates as well as inhibitors (Teixeira and Borges, 2012).

Functions were to mantain constant levels of ATP and ADP, buffering the cell against rapid depletion of ATP (Michael et al.; 2010).

2.3.1.3Diagnostic Significance

Because of the high concentrations of CK in muscle tissue, CK levels are frequently elevated in disorders of cardiac and skeletal muscle. The CK level is considered a sensitive indicator of acute myocardial infarction (AMI) and muscular dystrophy, particularly the Duchenne type. Striking elevations of CK occur in Duchenne-type muscular dystrophy, with values reaching 50 to 100 times the upper limit of normal (ULN). Although total CK levels are sensitive indicators of these disorders, they are not entirely specific indicators inasmuch as CK elevation is found in various other abnormalities of cardiac and skeletal muscle. Levels of CK also vary with muscle mass and, therefore, may depend on gender, race, degree of physical conditioning, and age. Elevated CK levels are also occasionally seen in central nervous system disorders such as cerebrovascular accident, seizures, nerve degeneration, and central nervous system shock. Damage to the blood–brain barrier must occur to allow enzyme release to the peripheral circulation.

Other pathophysiologic conditions in which elevated CK levels occur are hypothyroidism, malignant hyperpyrexia, and Reye's syndrome (Michael et al.; 2010).CK occurs as a dimer consisting of two subunits that can be separated readily into three distinct molecular forms. The three isoenzymes have been designated as CK-BB (brain type), CK-MB (hybrid type), and CK-MM (muscle type). On electrophoretic separation, CK-BB will migrate fastest toward the anode and is therefore called CK-1. CK-BB is followed by CK-MB (CK-2) and, finally, by CK-MM (CK-3), exhibiting the slowest mobility (Michael et al.; 2010).

2.4 Body mass index (BMI):

A calculation based on height and weight, is an indicator of total body fat. Extremes of total body fat are related to the development of cardiovascular

disease, diabetes, cancer, andother chronic disorders. Body mass index (BMI) is calculated as the weight in kg divided by the height in metres squared (kg/m2). Ideal body mass index is considered to be between 20 and 25(Wendy and Jean., 2007) .

Overweight, with a body mass index of greater than 25. Body mass index of greater than 29 considered as obesity (Wendy and Jean. 2007).

2.5 Blood pressure:

Blood Pressure is the Amount of Blood Pumped by the heart in relation to size and condition of arteries. Measured by Force of Blood on Artery Walls; in Millimeters of Mercury (mmHg).

Blood pressure measure = systolic pressure over diastolic pressure

(120/80 mmHg; Healthy Measurement.)

Blood pressure varies from moment to moment. It is affected by many factors including breathing, body position, emotional state, exercise, sleep, medicines and alcohol. (NHF, 2016).

Blood pressure may vary substantially and is affected by emotions, pain, eating, voiding, caffeine, nicotine, drugs, body position, mental activity and physical activity. Therefore, if an elevated blood pressure reading has been obtained, the blood pressure level should be confirmed on a different day. Confirmation may be based on one or more follow-up visits with at least two blood pressure readings at each visit, 24-hour ambulatory blood pressure monitoring or 3) home blood pressure monitoring using a validated device. causes of high blood pressure**:** Family history **,** Being overweight **,**Poor diet **,**Too much salt, Drinking too much alcohol ,Not exercising **,**Cigarette smoking **,**and Kidney problems. The risks of high blood pressure are: Heart disease, Heart failure, Stroke, Kidney disease or failure, and Eye problems (NHF, 2016).

Chapter three

Materials and methodology

Chapter three

Materials and methodology

3.1 Study approach:

Quantitative methods were carried out to evaluate the effect of wet blood cupping on CRP and CK level, pre and post for both. Among Sudanese people at Khartoum cupping centers from February to June 2017.

3.2 Study design:

Experimental study.

3.3 Study area:

This study was conducted at al-Khartoum state; in International center for blood cupping and prophet medicine, and Rwabih organization for social services.

3.4 Study population and Sample size:

The study included Sudanese volunteers whom blood cupping is carried on at Khartoum cupping centers. 40 samples were obtained; both male and female were included, their age between 20-70 years.

3.5 Ethical considerations:

The study was approved from clinical chemistry department and medical laboratory sciences- collage of graduated studies; in Sudan University for science and technology. Verbal informed consent was obtained.

3.6 Data collection:

Data were collected using structural interviewing questionnaire (Appendix I), which was designed to collect and maintain all valuable information concerning each case examined.

3.7 Samples collection and processing:

Samples were collected under aseptic conditions by using of alcohol 70% swabs , then blood was taken from the each volunteer at rest position ,5 ml

for the sample was collected following the standard procedures of blood collection ,this blood was taken into lithium heparin containers ,which were being centrifuged at 3000 rpm for 10 min ;this to obtain clear plasma , which being refrigerated at -20 until the time of analysis. This processing is identically done for both before and after cupping samples.

3.8 Methodology:

The laboratories tests were performed at Eljaili Khalid Musa Laboratory at Omdurman city, by using BTs-350 semi-automated chemical analyzer which based on the same principles of ordinary spectrophotometers with more develop in sample processing and rang of wavelengths and reading procedures.

3.8.1 C reactive protein:

3.8.1.1 Principle:

Serum CRP causes agglutination of latex particles coated with anti-human CRP .the agglutination of the latex particles is proportional to the CRP concentration and can be measured by turbidimetry.

3.8.1.2 Procedure of measurement :(appendix II).

3.8.2 Creatine kinase:

3.8.2.1 Principle:

Creatine kinase (CK) catalyzes the phosphorylation of ADP, in the presence of creatine phosphate to form ATP and creatine. The catalytic concentration is determined from the rate of NADPH formation measured at 340 nm, by means of hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6P-DH) coupled reactions.

3.8.2.2 Procedure of measurement: (appendix II).

3.9 Quality control:

The precision and accuracy of all methods used in this study were checked by commercially prepared pathological control sample before the application of the tests measurement.

3.10 Statistical analysis:

Data was analyzed to obtain means, standard deviation, and correlation by using statistical package for social science (SPSS) computer programmed version 22, T- test and Person's correlation were used for comparison and correlation.

Chapter four

Results

Chapter four

Results

The results of biochemical parameters; creatine kinase and C reactive protein for people who had blood cupping are given in tables and figures :

Table (4-1): articulate the mean concentration of plasma creatine kinase, C reactive protein, and blood pressure before and after blood cupping. The level of CRP was significantly decreased in post cupping compared to pre cupping samples of the same volunteers with p value 0.01, (mean ±SD:29.48 ±44.97 versus 17.88±27.71 mg/L respectively). While the level of CK was insignificantly different in post cupping compared to pre cupping samples of the same volunteers with p value 0.45, (mean ±SD:80.35±56.38 versus 87.70 ±56.7 U/L respectively).The blood pressure was significantly decreased in post cupping compared to pre cupping samples of the same volunteers with p value 0.00,(mean ±SD:103.06 ±13.13versus 98.03±11.74 mmHg respectively).

Table (4:2): Articulate the person correlation (r, p-value) ;showed that there was: positive correlation of age with BMI(r=0.42,p-value: 0.01), and also positive correlation of age with post cupping CRP(r=0.328, p-value:0.04), negative correlation of age with pre cupping CK (r=-0.349, p-value: 0.03), while positive correlation of BMI with pre Bp(, r=0.387, p-value: 0.04).

Figure (4.1): Shows no correlation between the post CRP and post CK (r= -0.223, p value 0.184).

Figure (4.2) shows no correlation between the pre CRP and pre Ck (r= -0.239, p value 0.155).

Figure (4.3) shows positive correlation between the pre BP and BMI (r= 0.387, p value 0.042).

Figure (4.4): shows positive correlation between the post Age and post BMI (r= 0.420, p value: 0.014).

Table (4-1): articulate the mean concentration of plasma creatine kinase, C reactive

Variables	Pre cupping	Post cupping	P. values
	Mean ± SD	Mean ± SD	
Creatine kinase U/L	80± 56.4	87± 56.7	0.45
C reactive protein mg/L	29.4± 45	17± 28	0.01
BP mm.Hg	103± 13	98± 11.7	0.00

protein, and blood pressure before and after blood cupping

*Result given in mean ±SD, ***P-Value*** ≤ 0.05 Consider significant.

*Paired sample T test was used for comparison.

Table (4:2): Articulate the person correlation (*p-value,* r) of age with BM, pre and post cupping CRP, BP , and CK. BMI with pre and post BP:

Variable	P Value	Person correlation (r)
CK with CRP in pre cupping	0.155	-0.239
CK with CRP in post cupping	0.184	-0.223
Age with BMI	0.014	0.420
Age with pre CK	0.034	-0.349
Age with post CRP	0.048	0.328
BMI with pre BP	0.042	0.387

*results given as value and person correlation.

* P-Value ≤ 0.05 Consider significant.

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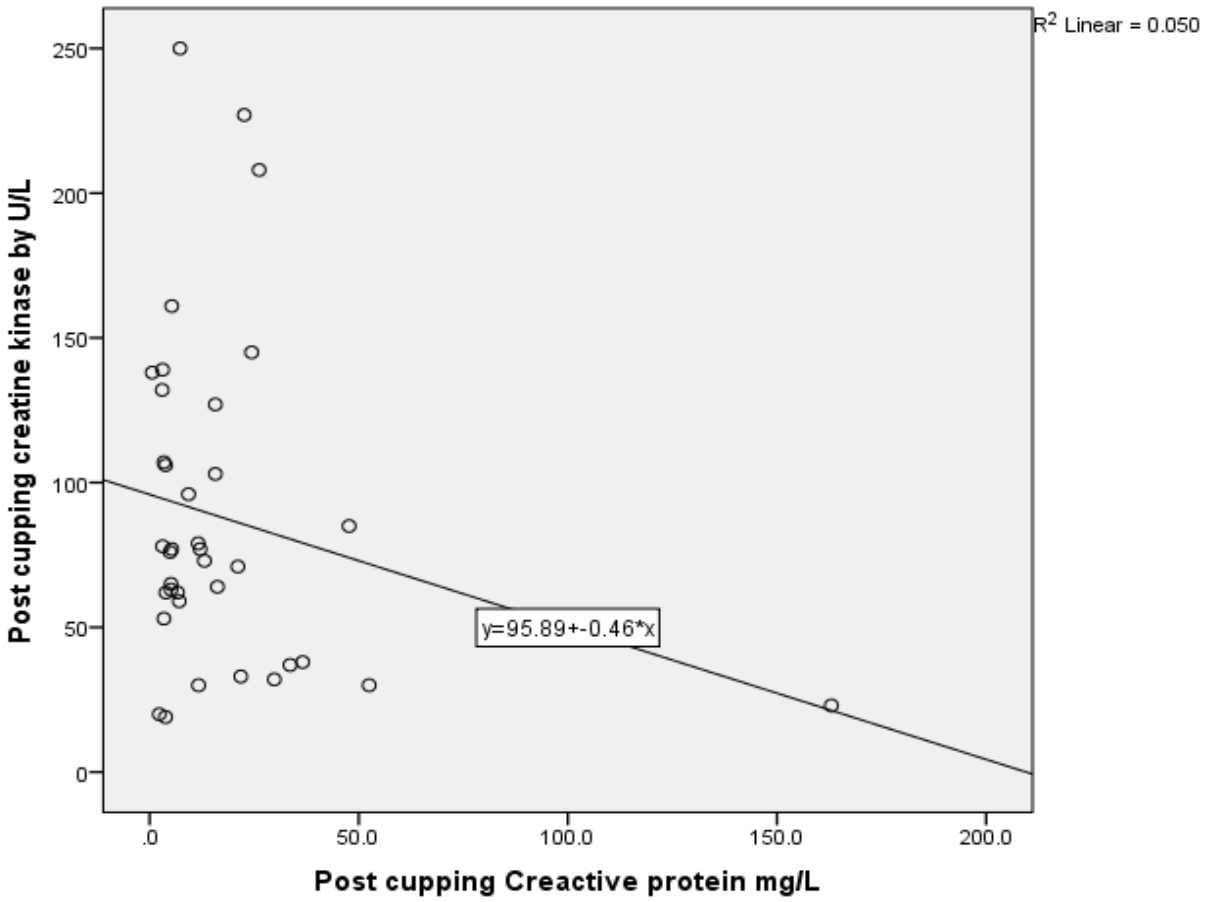

Figure (4.1) Correlation between the post CRP and post CK (r= -0.223, p value 0.184).

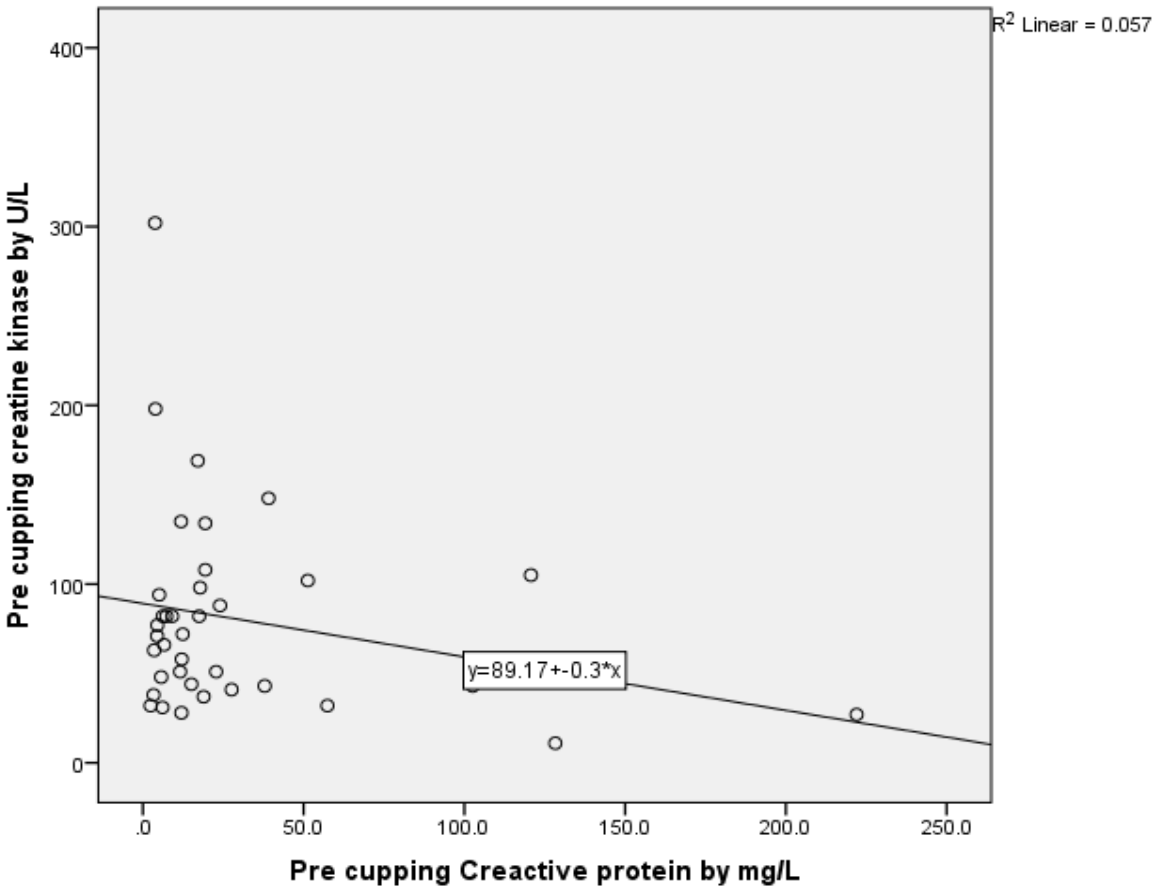

Figure (4.2) Correlation between the pre CRP and pre CK (r= -0.239, p value 0.155).

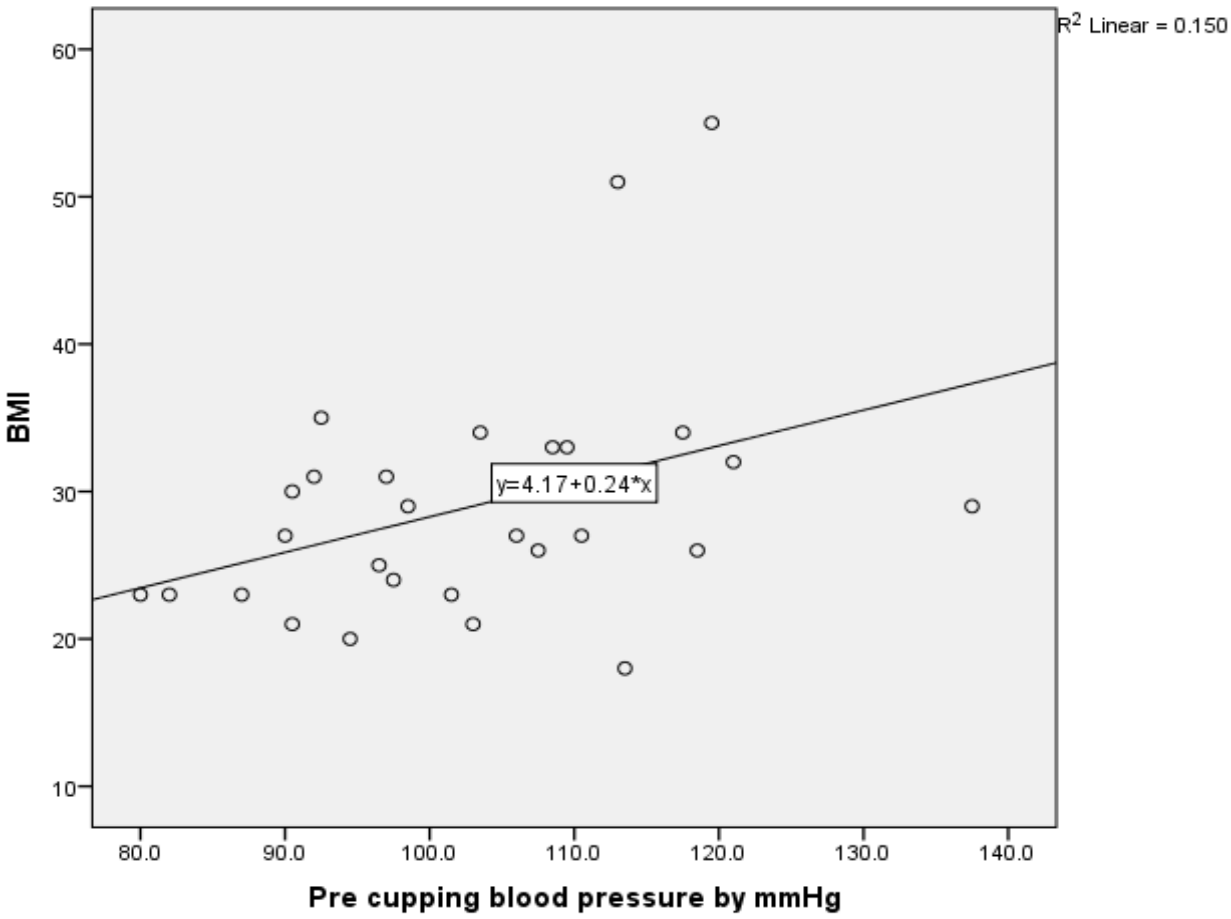

Figure (4.3) Correlation between the pre BP and BMI (r= 0.387, p value 0.042)

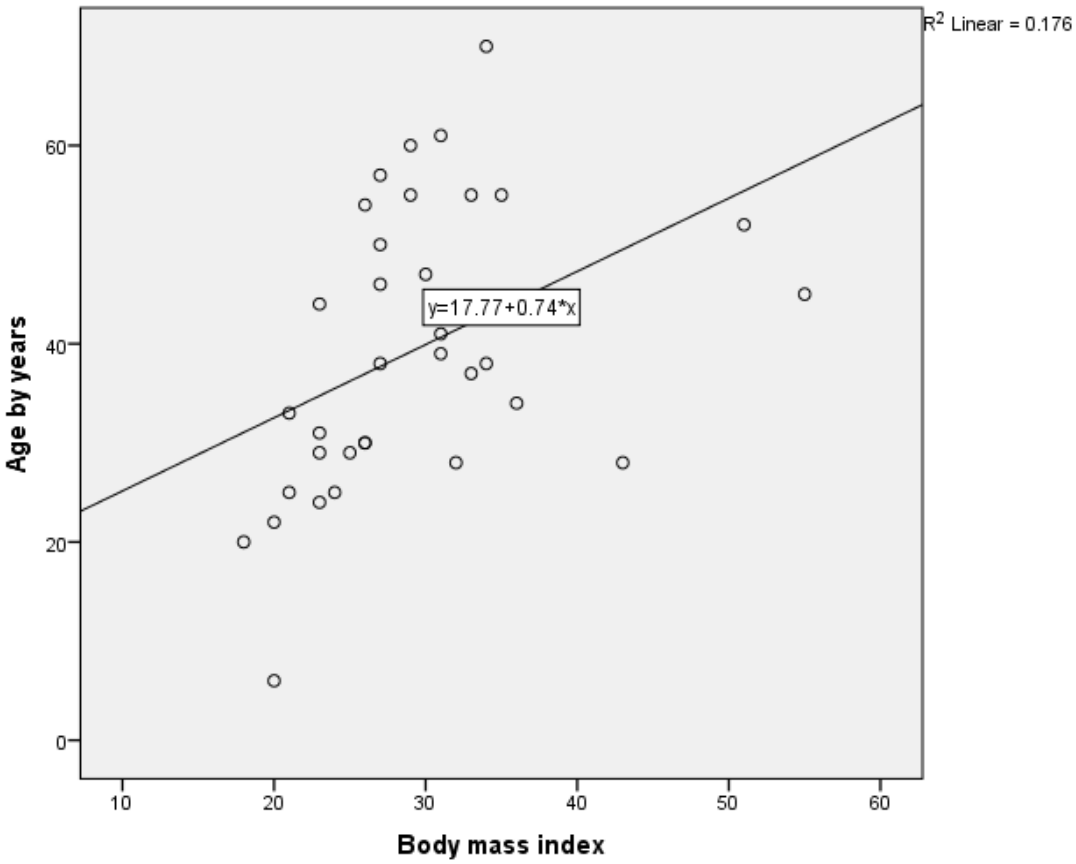

Figure (4.4): Correlation between the Age and BMI (r= 0.420, p value: 0.014).

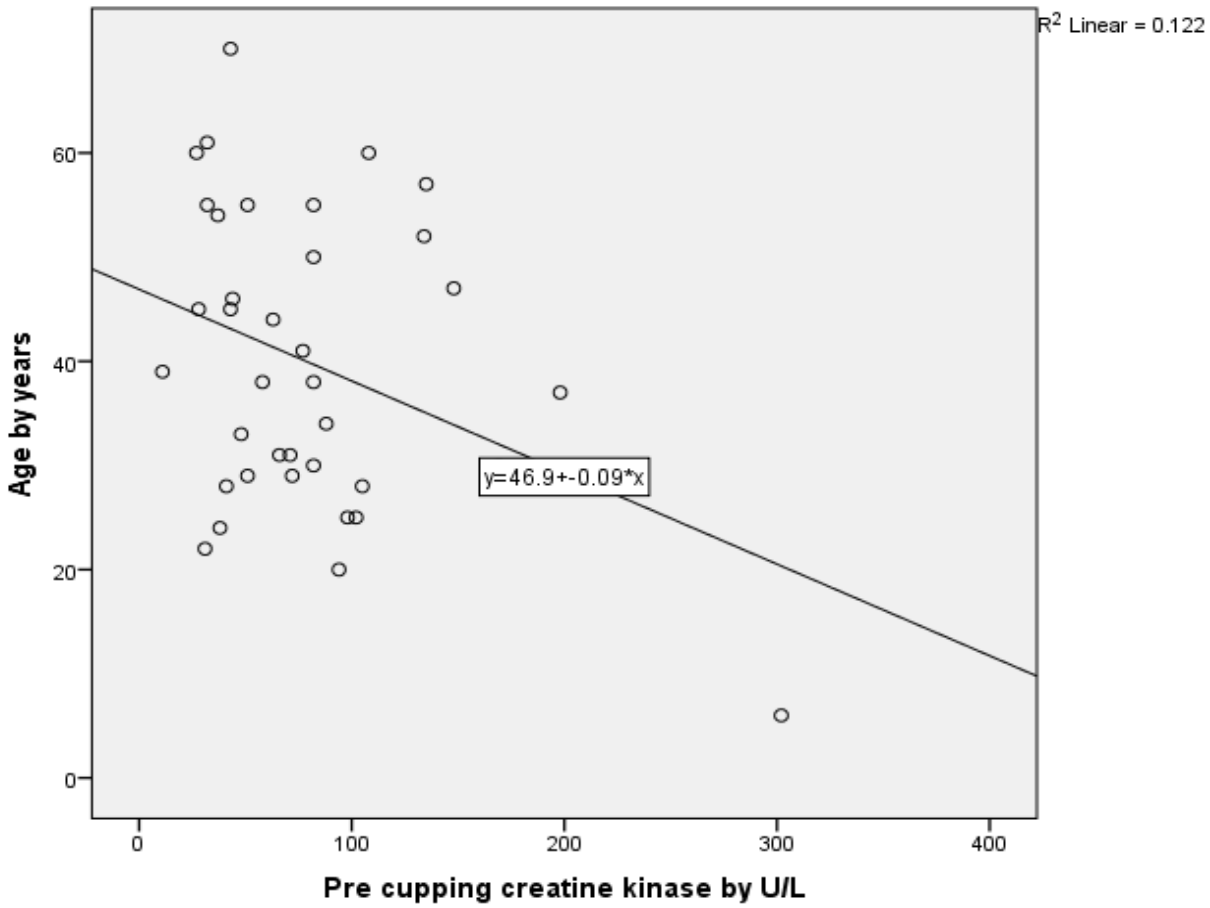

Figure (4.5) Correlation between the pre CK and Age (r= -0.349, p value 0.034)

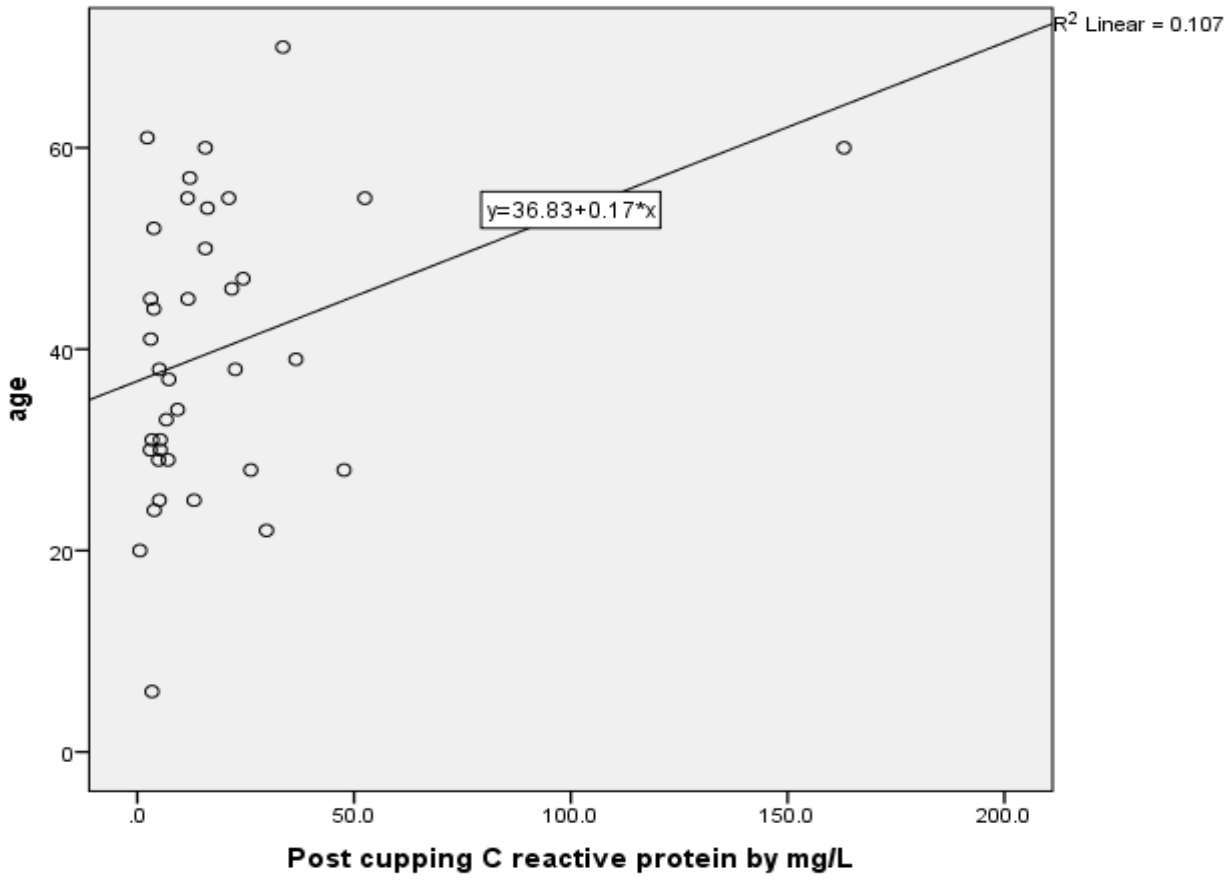

Figure (4.6) Correlation between the post CRP and Age (r= 0.328, p value 0.048)

Chapter five

Discussion& Conclusion and Recommendations

5.1 Discussion

Cupping belongs to the oldest medical procedures practiced in the human history and the therapeutic potential of cupping therapy is unlimited and the secrets of its mechanisms still mysteriouas (Bassem et al., 2014).

The Prophet Mohammad (peace be upon him) said: “If there is a benefit in any of your treatment modalities, benefit will be in the blade puncture in cupping therapy” According to an anecdote of Islams` Messenger advised that one should undergo cupping on 17, 19, and 21 not on 13, 14, and 15 day of every month of lunar calendar, when the moon gravitational is on the highest level, blood comes to the body surface and skin. Scientific facts in modern medicine agree with knowledge gained from prophetic medicine. This study samples collected at these days following the Islamic cupping protocol.

Despite the prominent role of modern medicine in diagnosis and management of many diseases, most of the times it is unable to control and prevent chronic disease. Many researchers have proven the efficacy of complementary and alternative medicine in this regard and such medical aspect is considered as integrative medicine (Hashem et al., 2008). Many questions arise from time to time about the exact role of cupping in treating diseases and to explain benefits of cupping therapy. It was suggested that cups produce hyperemia or hemostasis which results in a therapeutic effect (Cao et al, 2012).

This study was carried out to measure the blood level of creatine kinase and C reactive protein pre and post the cupping, venous blood samples was collected from forty volunteers aged 20-70 years.

The blood pressure statistical results showed significant decrease p-value 0.00, in the post cupping reading compared to the pre reading; the same result was obtained by study of (Bassem Refaat et al,2014) with difference that they calculated systolic and diastolic separately . This may be due to reduction of the increased volume of blood according to El Sayed et al (2013).

Elevated BP and hypertension are major risk factors for CVDs, especially CHD, stroke and heart failure, as well as renal failure. It has been estimated that high BP is involved in 49% of all chronic heart diseases and 62% of all stroke incidences. Nearly 50% of adults worldwide are estimated to be hypertensive or experiencing episodes of raised BP. More importantly, all currently used antihypertensive drugs, without exception, have several dose dependent side effects **(**Fairouz , 2010).

In the absence of other treatments of hypertension, blood cupping technique is said to promote blood circulation, remove stasis and could sometimes have had a beneficial effect in temporarily reducing blood pressure by a reduction in blood volume, including the fluid overload of heart failure **(**Fairouz ,2010) Therefore, hejamah is a potential therapy that could be used as prophylactic and/ or complementary treatment for the prevention and control of HTN.

The study results of CRP showed a significant decrease in the post cupping results comparing to pre one with p value 0.01 ,Hussam Baghdadi et al(2015) study also got the same finding, While Mahdavi et al (2011); showed no insignificant difference (p=0.121) in serum CRP level in comparison to venous blood samples.

C-reactive protein CRP is used mainly as a marker of acute inflammation. Change rapidly, either at the start of inflammation or as it goes away. Since

CRP is not affected by many other factors as is ESR does, making it a better marker of inflammation and measuring CRP values can be useful in determining disease progress or effectiveness of treatments.

The findings of this study showed that the levels of creatine kinase insignificantly different; p-value 0.44, this disagreed with the study of Mohammad Khalil et al(2016) that significantly increase ;this differences may be due to days of post cupping collections and sample size they used .

The increase may be due to the ruptured cells which contain the enzyme during cupping process.

Creatine kinase is often used as an indirect indicator of muscle damage and its release from muscle tissue into the blood is accompanied by cell membrane rapture. The intensity of muscle damage is related to both intensity and duration of activity. However, the intensity of activity plays a more important role.

The study results showed a positive correlation between age and BMI with p-value: 0.01, and person correlation; r=0.42. Also there was a positive correlation between the age and post cupping CRP with p-value: 0.04, and person correlation; r=0.328. while there was a negative correlation between the age and pre cupping CK with p-value: 0.03and person correlation; r=-0.349. The study results showed no correlation between the post CRP and post CK (r= -0.223, p value 0.184). Also there was no correlation between the pre CRP and pre Ck (r= -0.239, p value 0.155).

5.2 Conclusion:

The findings of this study indicating that blood cupping could decrease the level of the C reactive protein and blood pressure, while increase the level of creatine kinase.

5.3 Recommendations

- We recommend searching about cupping effects on patients with specific diseases for example CVDs.
- These results are presented after one or more time cupping with duration of 10-14 days, we offer planning of a proposal about doing cupping in different intervals to have a good investigation of cupping Time-Response.
- Hence the hormone playing an important role in controlling the body vital processes measuring the effect of Islamic wet cupping on the serum hormones levels such as aldosterone, cortisol, adrenaline and thyroid hormones could provide a better understanding on the underlying mechanism(s) by which it could affect the metabolic parameters.

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Appendices

Appendix I

Questionnaire

Sudan University of science and Technology

College of graduate studies

C reactive protein and Creatine kinase level in pre and post cupping

Name:……..
Patient Number : ( ).
Phone Number :( ).
Weight : ( ) Kg.
Height :( ) meters.
Age : ( )years.
BMI :( ).
Pre blood pressure :( ).
Post blood pressure : ( ).

Laboratory investigations:-

Test name	Concentration
Creatine kinase	U/L
C reactive protein	mg/L

Appendix II

Contain the leaflet both CRP &CK

COD 11790 1 x 50 mL	COD 11791 4 x 50 mL
STORE AT 2-8ºC	
Reagents for measurement of CK concentration Only for *in vitro* use in the clinical laboratory	

CREATINE KINASE (CK)

BioSystems
REAGENTS & INSTRUMENTS

CE

CREATINE KINASE (CK)
IFCC

PRINCIPLE OF THE METHOD

Creatine kinase (CK) catalyzes the phosphorylation of ADP, in the presence of creatine phosphate, to form ATP and creatine. The catalytic concentration is determined from the rate of NADPH formation, measured at 340 nm, by means of the hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6P-DH) coupled reactions[1,2].

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{Glucose} \xrightarrow{\text{HK}} \text{ADP} + \text{Glucose - 6 - phosphate}$$

$$\text{Glucose - 6 - phosphate} + \text{NADP}^+ \xrightarrow{\text{G6P-DH}} \text{6 - Phosphogluconate} + \text{NADPH} + \text{H}^+$$

CONTENTS

	COD 11790	COD 11791
A. Reagent B. Reagent	1 x 40 mL 1 x 10 mL	4 x 40 mL 4 x 10 mL

COMPOSITION

A. Reagent: Imidazol 125 mmol/L, EDTA 2 mmol/L, magnesium acetate 12.5 mmol/L, D-glucose 25 mmol/L, N-acetyl cysteine 25 mmol/L, hexokinase 6000 U/L, NADP 2.4 mmol/L, pH 6.7.

B. Reagent: Creatine phosphate 250 mmol/L, ADP 15 mmol/L, AMP 25 mmol/L, P1,P5-di(adenosine-5'-)pentaphosphate, 102 µmol/L, glucose-6-phosphate dehydrogenase 8000 U/L.

STORAGE

Store at 2-8ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagents: Presence of particulate material, turbidity, absorbance of the blank over 0.300 at 340 nm (1 cm cuvette).

REAGENT PREPARATION

Working Reagent: Pour the contents of the Reagent B into the Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B.

Stable for 15 days at 2-8ºC. The working reagent must be protected from light.

ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer with cell holder thermostatable at 25, 30 or 37ºC and able to read at 340 nm.

– Cuvettes with 1 cm light path.

SAMPLES

Serum and plasma collected by standard procedures.

Creatine kinase in serum and plasma is stable for 7 days at 2-8ºC. Use heparin or EDTA as anticoagulant.

PROCEDURE

1. Bring the Working Reagent and the instrument to reaction temperature.
2. Pipette into a cuvette: (Note 1)

Sample	50 µL
Working Reagent	1.0 mL

3. Mix and insert the cuvette into the photometer. Start the stopwatch.
4. After 3 minutes, record initial absorbance and at 1 minute intervals thereafter for 3 minutes.
5. Calculate the difference between consecutive absorbances, and the average absorbance difference per minute (ΔA/min).

CALCULATIONS

The CK concentration in the sample is calculated using the following general formula:

$$\Delta A/\text{min} \times \frac{Vt \times 10^6}{\varepsilon \times l \times Vs} = \text{U/L}$$

The molar absorbance (ε) of NADPH at 340 nm is 6300, the lightpath (l) is 1 cm, the total reaction volume (Vt) is 1.05, the sample volume (Vs) is 0.05, and 1 U/L are 16.67 nkat/L. The following formulas are deduced for the calculation of the catalytic concentration:

ΔA/min	x 3333 = U/L x 55561 = nkat/L

REFERENCE VALUES

Reaction Temperature	Men[3] U/L	Men[3] nKat/L	Women[3] U/L	Women[3] nKat/L
25ºC	10-65	167-1084	7-55	117-917
30ºC	15-105	250-1750	10-80	167-1334
37ºC	38-174	633-2900	26-140	433-2334

Children have higher CK concentrations than adults[3]. These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit: 9.2 U/L = 153 nkat/L

– Linearity limit: 1300 U/L = 21671 nkat/L. For higher values dilute sample 1/2 with distilled water and repeat measurement.

– Repeatibility (within run):

Mean Concentration	CV	n
175 U/L = 2917 nkat/L	1.8 %	20
567 U/L = 9452 nkat/L	0.7 %	20

– Reproducibility (run to run):

Mean Concentration	CV	n
175 U/L = 2917 nkat/L	1.3 %	25
567 U/L = 9452 nkat/L	1.1 %	25

– Sensitivity: 0.3 ΔmA·L/U·min = 5 ΔmA·L/nkat·min

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

– Interferences: Bilirubin (< 20 mg/dL) and hemoglobin (< 10 g/L) do not interfere. Lipemia (triglycerides > 5 g/L) interfere. Other drugs and substances may interfere[4].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Creatine kinase (CK) plays an important role in muscle by providing ATP, when muscle contracts, from ADP and using creatine phosphate as the phosphorylation reservoir.

Serum CK originates mainly in muscle and its concentration is subject to a number of physiological variations (sex, age, muscle mass, physical activity and race).

Serum CK concentration is greatly elevated in patients with some diseases of skeletal muscle (muscular distrofy, myositis, polymyositis, malignant hyperthermia, trauma, acute rhabdomyolysis), of the central nervous system (acute cerebrovascular disease, cerebral ischemia, Reye's syndrome) and of the thyroid (hypothyroidism)[3,5].

After a myocardial infarction, CK elevation begins in 3-6 hours and peaks at 24-36 hours. The enzyme is rapidly cleared from the plasma, so that it is common for the activity to return to normality in 3-4 days[3,5].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.

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COD 31321 1 x 20 mL	COD 31921 1 x 50 mL	COD 31029 2 x 200 mL
STORE AT 2-8ºC		
Reagents for measurement of CRP concentration Only for *in vitro* use in the clinical laboratory		

C-REACTIVE PROTEIN (CRP)

BioSystems REAGENTS & INSTRUMENTS

CE

C-REACTIVE PROTEIN (CRP)
LATEX

PRINCIPLE OF THE METHOD

Serum C-reactive protein (CRP) causes agglutination of the latex particles coated with anti-human C-reactive protein. The agglutination of the latex particles is proportional to the CRP concentration and can be measured by turbidimetry[1-4].

CONTENTS

	COD 31321	COD 31921	COD 31029
A. Reagent	1 x 16 mL	1 x 40 mL	2 x 160 mL
B. Reagent	1 x 4 mL	1 x 10 mL	2 x 40 mL

COMPOSITION

A. Reagent: Glycine buffer 0.1 mol/L, sodium azide 0.95 g/L, pH 8.6.

B. Reagent: Suspension of latex particles coated with anti-human CRP antibodies, sodium azide 0.95 g/L.

STORAGE

Store at 2-8ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagents: absorbance of the blank over 0.900 at 540 nm.

AUXILIARY REAGENTS

S. CRP Standard: 1 x 1 mL (BioSystems Cod. 31113). Human serum. C-reactive protein concentration is stated on the vial label. The concentration value of the CRP Standard is traceable to the ERM-DA472/IFCC (Institute for Reference Materials and Measurements, IRMM).
Human serum used in the preparation of the standard has been tested and found to be negative for the presence of antibodies anti-HIV and anti-HCV, as well as for HBs antigen. However, the standard should be handled cautiously as potentially infectious.

Reconstitute with 1.0 mL of distilled water. Stable for 1 month at 2-8ºC.

REAGENT PREPARATION

Working Reagent: Pour the contents of a Reagent B vial into a Reagent A bottle (Note 1). Mix thoroughly. Stable for 60 days at 2-8ºC.

Smaller Working Reagent volumes can be prepared by mixing: 1 mL of Reagent B + 4 mL of Reagent A. Shake the Reagent B vial before pipetting.

ADDITIONAL EQUIPMENT

– Thermostatic water bath at 37ºC

– Analyzer, spectrophotometer or photometer thermostatable at 37ºC able to read at 540 ± 20 nm.

SAMPLES

Serum collected by standard procedures.

CRP in serum is stable for 7 days at 2-8ºC.

PROCEDURE

1. Bring the Working Reagent and the instrument to 37ºC.
2. Zero the instrument with distilled water (Note 2)
3. Pipette into a cuvette:

Working Reagent	1.0 mL
Standard (S) or Sample	7 µL

4. Mix and immediately insert cuvette into the instrument. Start stopwatch.
5. Record the absorbance at 540 nm after 10 seconds (A_1) and after 2 minutes (A_2).

CALIBRATION

A calibration is recommended at least every 2 months, after reagent lot change or as required by quality control procedures.

CALCULATIONS

The CRP concentration in the sample is calculated using the following general formula:

$$\frac{(A_2\text{-}A_1)_{\text{Sample}}}{(A_2\text{-}A_1)_{\text{Standard}}} \times C_{\text{Standard}} = C_{\text{Sample}}\ (\text{mg/L})$$

REFERENCE VALUES

Serum, adults[5]: Up to 5 mg/L.

This range is given for orientation only; each laboratory should establish its own reference range.

QUALITY CONTROL

It is recommended to use the Rheumatoid Control Serum level I (Cod. 31213) and II (Cod. 31214) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit: 1.0 mg/L.

– Linearity limit: 150 mg/L. For higher values dilute sample 1/5 with distilled water and repeat measurement. Linearity limit may vary depending on the photometer or analyzer used. (Note 3)

– Repeatibility (within run):

Mean concentration	CV	n
7.4 mg/L	4.5 %	20
19.0 mg/L	3.6 %	20

– Reproducibility (run to run):

Mean concentration	CV	n
7.4 mg/L	4.6 %	25
19.0 mg/L	3.7 %	25

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

– Zone effect: This method has not zone effect (< 500 mg/L).

– Interferences: Hemoglobin (10 g/L), bilirubin (20 mg/dL), lipemia (triglycerides 10 g/L) and rheumatoid factors (200 IU/mL) do not interfere. Other drugs and substances may interfere[6].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

C-Reactive Protein (CRP), which is synthesized in the liver, is one of the the most sensitive acute phase reactants after tissue damage or inflammation. CRP activates the classical complement pathway as a response to the inflammatory reaction.

CRP levels in plasma can rise dramatically after myocardial infarction, stress, trauma, infection, inflammation, surgery or neoplastic proliferation. The increase occurs within 24 to 48 hours and the level may be 2000 times normal. An elevation can be expected in virtually all diseases involving tissue damages so the finding is nonspecific[7].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. Shake the Reagent B vial gently before pouring its contents into the Reagent A bottle. It is advisable to wash the Reagent B vial with a small volume of the prepared mixture in order to completely rinse the vial and avoid any losses.
2. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.
3. The linearity limit depends on the sample to reagent ratio. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.

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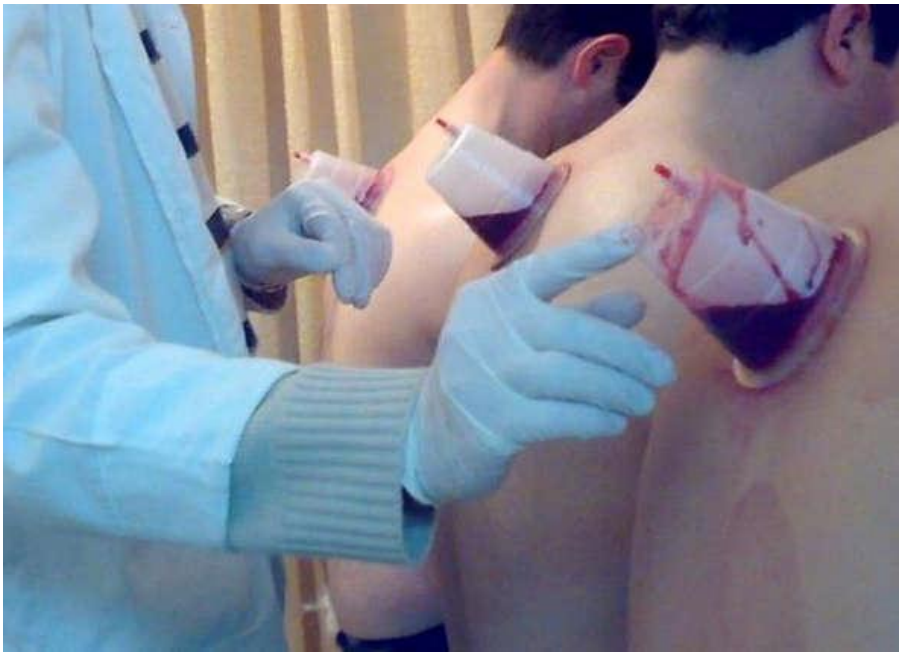
Appendix III

These pictures explain the tools of blood cupping



Appendix IV

Jaabir ibn Abdullaah (RA) reported hadeeth that reported by Saheeh Sunan ibn Maajah (2807).

Salma (RA) reported hadeeth that reported by Saheeh Sunan abi Dawud (3858).

Ibn al-Qaiyum (RA) reported hadeeth that reported by Zaad al Ma'aad (4/125-126).

Abdullaah ibn Abbas (RA) reported hadeeth that reported by Ahmed (1/305)