بسم الله الرحمن الرحيم

Sudan University of Science & Technology

College of Graduate Studies

Effect of Blood Cupping (Al-hijama) on Total Cholesterol, High Density Lipoprotein cholesterol and Low Density Lipoprotein cholesterol levels

تأثير الحجامة على مستويات الكوليسترول الكلي وكوليسترول البروتين الدهني عالي الكثافة و وكوليسترول البروتين الدهني منخفض الكثافة

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الأية

قال تعالى:

(ولو أنما فى الأرض من شجرة أقلام والبحر يمده من بعده سبعة أبحر
ما نفدت كلمات الله إن الله عزيز حكيم)

صدق الله العظيم

سورة لقمان الأية (27)

Dedication

I dedicate this modest for

My parents

Source of my life and pulse of my heart

My brothers and sisters

Reason of my happiness in my life

For my husband

Who helps me to pass the difficulty of my life

My teachers

The reason of advancement and success

For all my friends and my lovely people who have role in my life ….to them I dedicate my accomplishment.

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Complete this research.

Abstract

The word "hijama" is derived from "hajm" which means "sucking." cupping is the process of applying cups to various points on the body by removing the air inside the cups to form a vacuum.

This study was carried out in Al- Almi for alternative medicine, Al-Rawabih charity organization for Al-Hijamah and Al-Madeina Al-Monwara clinic in Khartoum state; from April to August (2017), to detect the effect of Blood Cupping on Total Cholesterol, High density lipoprotein cholesterol (HDL-c) and Low Density Lipoprotein cholesterol (LDL-c); by measuring of their levels before and after 10 days of the Blood Cupping done respectively. This study also aimed to find out if there is a correlation between above parameters before and after cupping done. Forty (40) subjects with different age groups were informed about the nature of study and their agreement was obtained. 2.5 ml venous blood sample was collected before and 10 days after the Blood Cupping which then put in heparine container; plasma sample was separated using centrifuge, the concentrations of the parameters are determined twice time before and after 10 days of Blood Cupping by using semi automated mindray instrument.

The results obtained and analyzed using software program (SPSS) version 21; to give the means ± SD concentrations in both before and after 10 days of blood cupping. The result showed that there was a significant decrease in Total Cholesterol and LDL-c parameters before and after 10 days of blood cupping (177± 44.5 to 161 ± 37.3) mg/dl and (113 ± 44.2 to 93 ± 41.2) respectively; however the HDL-c parameter show a significant increase before and after 10 days of blood cupping (46 ± 18.1 to 53 ± 22.7) mg/dl.

Also this study found that there was a significant decrease in the mean of blood pressure after 10 days of blood cupping (122 ± 27.7 to 118 ± 24.6). Additionally this result found that there was a strong positive correlation between mean of blood pressure pre and post 10 days of blood cupping and moderate positive correlation between Total Cholesterol, HDL-c and LDL-c parameters pre and post blood cupping.

مستخلص البحث

الحجامة مشتقة من كلمة (الحجم) وتعنى مص وهى عملية سحب دم المريض في عدة مناطق مختلفة بإستخدام كوؤس ، وذلك بفصده بعد تفريغ الهواء داخل الموجود بداحل الكوؤس.

أجريت هذه الدراسة بمركز العالمي للطب البديل،منظمة الروابح الخيرية للحجامة ومركز المدينة المنورة الطبي بولاية الخرطوم؛ في الفترة الزمنية أبريل إلى أغسطس (2017) لمعرفة تأثير الحجامة علي معدل تراكيز الكوليستيرول الكلي، كوليستيرول البروتين الدهني عال الكثافة (الكوليسيرول الجيد) وكوليستيرول البروتين الدهني منحفض الكثافة (الكوليسيترول الضار)؛ وذلك بقياس معدلاتها قبل وبعد مرور 10 أيام من الحجامة؛ أيضا تهدف هذه الدراسة لإيجاد العلاقات بين تراكيز هذه المقاييس قبل وبعد الحجامة. أخذت 40 عينة من فئات عمرية مختلفة عشوائيا بعد أخذ موافقتهم على المشاركة فى هذا البحث، تم أخذ 2.5 مل من عينة الدم قبل وبعد 10 أيام من الحجامة من كل فرد ومن ثم تم وضعها مباشرة في حاوية مانع التجلط ؛ تم فصل البلازما بإستخدام جهاز الطرد المركزي ومن ثم قياس التراكيز بإستخدام جهاز المطياف الضوئي. تم إستخدام الحزم الإحصائية بإصدار رقم 21 لتحليل النتائج ومعرفة متوسط معدل الزيادة أو النقصان في التركيز قبل وبعد مرور 10 أيام من الحجامة.

أوضحت النتائج أن هناك نقصان ذو دلالة إحصائية في قياس تراكيز الكوليستيرول الكلي من (44.5 ±177إلي 37.3±161) و كوليستيرول البروتين الدهنى منخفض الكثافة من (44.2±113 إلي 93 ± 41.2). بينما هناك زيادة ذات دلالة إحصائية في تركيزكوليستسرول البروتين الدهن عال الكثافة بعد إجراء الحجامة من (46 ± 18.1إلي 53 ± 22.7).

كما أوضحت هذه الدراسة أن هناك نقصان ذو دلالة إحصائية في معدل متوسط ضغط الدم من 27.7 ± 122 إلي 24.6 ±118 ، كما وجد أن هناك علاقة إيجابية قوية في معدل النقصان في متوسط ضغط الدم قبل وبعد 10 أيام من الحجامة وعلاقة إيجابية متوسطة في معدل نقصان الكوليستيرول الكلي ، كوليسترول البروتين الدهني منخفض الكثافة و علاقة إيجابية متوسطة في معدل زيادة كوليستيرول البروتين الدهني عال الكثافة.

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Chapter One

1.1Introduction:

The Cupping process is considered as an alternative treatment which was done before 1500 years BCE. The art of cupping has been practiced from ancient times. Cupping therapy is one of the medicines of the prophet Muḥ ammad Rasulullaah (Sallallaahu Álayhi Wasallam); Narrated by Abu Hurayrah: The Nabi (pbuhpbuh) said: The best medical treatment you apply is Hijamah. (Abu Dawud 22:3848). In addition Cupping was highly recommended by pioneers of Islamic Medicine such as Ibn Sīnā, al-Zahrāwi and al-Rāzi. These physicians demanded a more stringent rule of application and paid close attention to the practical aspects of the procedure, especially regarding timing, and the Physical and mental condition of the patient. (Manz Hedwig, 2009). Cupping therapy is a simple procedure in which negative pressure is applied to the skin through sucking cups (dry cupping therapy) (AL-Shamma, 2009). Many types of cupping therapy are described in the literature including dry cupping therapy, wet cupping therapy, medicinal cupping therapy, moving cupping therapy and others (Chirali, 1999). The most important type of wet cupping therapy seems to be Al-hijamah (method of wet cupping therapy practiced in prophetic medicine).

It can be used in the treatment of a sudden increase in blood pressure, and in discharging pus from boils and furuncles, which represents excess, with blood-heat and stagnation. Sterilize the selected points with alcohol and make a very small incision with a triangle-edged needle or, using a plum-blossom needle, firmly tap the point for a short time to cause bleeding. Second negative pressure suctioning completes the process of waste excretion (Cao H *et al*. 2012).

Unfortunately, medical research related to cupping therapy does not cover its importance. Cupping therapy is practiced officially in hospitals in China (Cao H *et al*. 2012) and is considered very familiar in some European countries (Michalsen A *et al*. 2009, Salomonsen LJ *et al*. 2011). Current methodologies used for cupping therapy differ according to the type of cupping therapy e.g. dry cupping method differs from medicinal cupping method (Cao H *et al.* 2010). Cupping used for internal disease and structural problems. This method in multiple forms spread into medicine in Asian and European civilizations.

Lipids and lipoproteins, which are central to the energy metabolism of the body, lipids consider the main source of body fuel; it have become increasingly important in clinical practice; the very low density lipoproteins (VLDL) are like tanker trucks, carrying triglycerides assembled in the liver to cells for energy needs or storage as fat. The low-density lipoproteins (LDL), rich in cholesterol, are the nearly empty tankers that deliver cholesterol to peripheral cells and liver after the triglycerides have been off-loaded. The high-density lipoproteins (HDL) are the cleanup crew, gathering upexcess cholesterol for transport back to the liver. Cholesterol is used by the body for such useful functions as facilitating triglyceride transport by lipoproteins, for maintaining the normal structure and integrity of cell membranes, and as a precursor for steroid hormone synthesis, but when in excess, it can lead to cardiovascular disease (Michael L *et al.* 2010).

1.2 Rationale:

Cupping, or Hijāmah, is a time-tested and effective sunnah for assisting in the removal of toxins from the body. It is a safe, inexpensive and effective practice for the treatment of a range of ailments, and in the relief of intractable pain. It has been an important part of Ṭ ibb al-Nabawī for centuries, and is used in several traditional medical systems. Abu Hurairah (RA) narrates that Rasulullaah (SAW) said: "Jibra'eel conveyed to me that the best amongst the things that mankind uses for treatment is hijamah" (Sahih Al-Jaami 213).

Previous studies showed that there was significant decrease in Total Cholesterol, Low density lipoprotein cholesterol (LDL-c) and significant increase in High density lipoprotein cholesterol (HDL-c) (Fairouz k, 2010). But there is no study for alhijamah done in Sudan so iam interest to do it and to see the veffect of wet blood cupping on Total cholesterol, HDL-c and LDL-c.

1.3 Objectives:

1.3.1General objective:

-To study the Effect of blood cupping on total Cholesterol, HDL-c and LDL-c levels.

1.3.2 Specific Objectives:

-To measure and compare Total Cholesterol before and after blood cupping.

-To measure and compare HDL-c before and after blood cupping.

-To measure and compare LDL-c before and after blood cupping.

- To measure and compare blood pressure before and after blood cupping.

-To see the frequencies of the diseases this came to be treated by Al-Hijamah.

Chapter Two

Literature Review

2.1 Hijama:

The Arabic word "*hijama*" means "sucking." In the Persian Gulf, Hijama was used not only for treatment but also for prophylaxis against diseases. The pearl divers in the Persian Gulf used to undergo hijama before the diving season in the belief that the procedure will prevent diseases during the 3 months at sea. It was thought to be very effective against dizziness (Reflections, 2004). Cupping refers to an ancient Chinese practice in which a cup is applied to the skin and the pressure in the cup is reduced (by using change in heat or by suctioning out air), so that the skin and superficial muscle layer is drawn into and held in the cup. In some cases, the cup may be moved while the suction of skin is active, causing a regional pulling of the skin and muscle (the technique is called gliding cupping).This treatment has some relation to certain massage techniques, such as the rapid skin pinching along the back that is an important aspect of tuina. In this practice, the skin is pinched sometimes at specific points (e.g., bladder meridian points), until a redness is generated (Nielsen, 1995). Common sites for cupping were the temples, behind the ears, the base of the spine and over the upper part of the back. The cups were domed, usually made of glass, and the technique involved the vacuum principle. Tissue paper, rag or alcohol was burned in the cup, which was then flipped over and applied to the skin. The vacuum sucked both tissue and blood into the cup. Later improvements involved using a valve and a pump, eliminating the heating mechanism (which often led to painful blistering) (Curtis, 1981). Wet cupping involved scarilfying (developed in the 17th century), the skin with a scarification which had from one to 16 blades.

The depth of the blade was regulated by a screw, or a spring known as a Spring Fleam or Schnapper. When the cup was applied to the scarified skin, the blood was drawn out through the wounds. Cupping was still used in the early 20th century, but only for drawing blood from poisoned wounds (Curtis, 1981). Cupping is applied to certain acupuncture points (especially on T2-T5 vertebrates), as well as to regions of the body that are affected by pain (where the pain is deeper than the tissues to be pulled). Heating of the cups was the method used to obtain suction: the hot air in the cups has a low density and, as the cups cool with the opening sealed by the skin, the pressure within the cups declines, sucking the skin into it. In this case, the cups are hot and have a stimulating effect something like that of burning moxa wool (Soyuncu, 2009).

2.1.2 Ahadeeth on Hijamah:

Jabir bin Abdullaah (RA) relates that he heard Rasulullaah (SAW) saying: "If there is any good in your treatments it is in the blade of the Hajjaam, a drink of honey or branding by fire (cauterization), whichever suits the ailment, and I do not like to be cauterized" (Bukhari and Muslim).

Narrated By Abu Hurayrah (RA): Abu Hind cuppedbthe Nabi (SAW) in the middle of his head. The Nabi (SAW) said: Banu Bayadah, marry Abu Hind (to your daughter), and ask him to marry (his daughter) to you. He said: The best thing by which you treat yourself is Hijamah (Abu Dawud 5:2097).

Abu Hurairah (RA) narrates that Rasulullaah (SAW) said: "Whoever has hijamah done on the 17th, 19th or 21st of the month, it will be for him a cure from every illness" (Sahih Al-Jaami' 5968).

Abdullaah ibn Abbas (RA) reported that the Rasul (SAW) said, "I did not pass by an angel from the angels on the night journey except that they all said to

me: Upon you is Hijamah, O Muhammad." [Saheeh Sunan ibn Maajah (3477)].

In the narration reported by Abdullaah ibn Mas'ud (RA) the angels said, "Oh Muhammad, order your ummah (nation) with Hijamah." [Saheeh Sunan Tirmidhi (3479)].

Rasulullaah (Sallallaahu Álayhi Wasallam) said, 'Jibraaeel (Álayhis salaam) repeatedly emphasized upon me to resort to Hijamah to the extent that I Feared that Hijamah will be made compulsory.' (Jamúl Wassail p. 179).

Rasulullaah (Sallallaahu Álayhi Wasallam) praised a person who performs Hijamah, saying it removes blood, lightens the back and sharpens theeyesight (Jamúl Wasaail p. 179).

2.1.3 History of cupping therapy:

Cupping therapy is being practiced nowadays in many countries all over the world including Germany, Norway, Denmark, Saudi Arabia, Egypt, India, China and other countries. German people are familiar with cupping therapy (Michalsen A *et al*. 2009) and so are Danish and Norwegian peoples where those European societies already have a shift in attitude to include complementary medicine within the conventional health care system (Salomonsen LJ *et al*. 2011). The exact origin of cupping therapy is a matter of controversy. Chinese scientists report in their literature that cupping therapy is a part of the traditional Chinese medicine (TCM) dating back to at least 2,000 years (Chirali IZ, 1999) In the middle east, Arabic writers report that cupping therapy dates back to 3500 B.C. (5500 years ago), where Assyrians were the first Arab population to use primitive tools as animal horns and bamboo wood for cupping therapy then the Chinese physician, Jee Hong (381- 281 B.C.) was among the leaders in that art (Shaheed Omar, 2009). Arabic civilization termed cupping therapy, Al-hijamah therapy (which means

in Arabic: to restore to the original size), where it was used in treating hypertension, polycythemia, headache, migraine and drug intoxication. They diagnosed polycythemia whenever there was an exaggeration of the pink color of the skin (Shaheed Omar, 2009). Interestingly, venesection (phlebotomy) is still being used currently in hospitals for treating polycythemia, where blood is drawn out and is replaced by saline infusion (McMullin MF *et al.* 2005) Ancient Egyptians were reported to practice cupping therapy earlier than many old civilizations, where cupping therapy was one of the oldest known medical therapies in ancient Egypt. The first report of using cupping therapy in ancient Egypt dates back to 1550 B.C. (more than 3500 years ago) where drawings on the famous Egyptian papyrus paper (Ebers paper) and ancient Egyptian temples showed that Egyptians were advanced in treatment using cupping therapy. Cupping therapy was also used in ancient Greek medicine (Christopoulou-Aletra H and Papavramidou N 2008, Teut M *et al*. 2012).

In 400 B.C., Herodotus (a Greek historian) recorded that the ancient Egyptian physicians who recommended the application of sucking cups to the body already used both wet cupping therapy and dry cupping therapy. Diseases treated by cupping therapy included headache, lack of appetite, maldigestion, fainting, abscess evacuation, narcolepsy (repeated sleepy desires) and others (Turk JL and Allen E, 1983). In 3300 BC, in Ancient Macedonia, cupping therapy had been used since prehistoric times to treat diseases and health disorders (Abele J, 1996) and (Eisenberg DM *et al.* 1998). In the United States, there is a progressive increase in the use of cupping therapy and other types of complementary medicine (Eisenberg DM *et al.* 1998) In a recent report from Harvard medical school about pediatric patients suffering from chronic severe pain, authors reported that cupping and acupuncture treatment were pleasant and helpful for pain treatment (Kemper KJ *et al.* 2000)

Currently, most widely used practice for cupping therapy is in China. Cupping therapy is considered by Chinese to be part of the TCM. Chinese hospitals recognized cupping therapy as a formal modality of treatment since 1950 (Cao H *et al*, 2012). Currently, medical practitioners in China and Mongolia are practicing cupping therapy for treating hypertension, neck pain, and headache, chronic hepatitis, ophthalmic diseases skin diseases and infectious diseases (Kim TH *et al*, 2011). Loukas et al. recently reported that knowledge gained from religious texts (Qur'an and Hadeeth) may guide attention of researchers to start research and get some benefits when comparing such knowledge with modern medical knowledge. Prophetic medicine is the medical knowledge gained from sayings, advices and teachings of Prophet Mohammad peace be upon him, (Loukas M *et al*, 2010, Al-Bukhari MI 1996) which recommended many lines of treatment as cupping therapy: (If there is a benefit in any of your treatment modalities, benefit will be in the blade puncture in cupping therapy, a gulp of honey and cauterizing, but I do not like cauterization (Loukas M *et al*, 2010, Al-Bukhari MI 1996).

2.1.4 Calcification of Cupping Therapy:

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 (Cao H *et al*. 2010).

The third is method of suction related category which includes fire, manual vacuum, and electrical vacuum cupping therapy (Cao H *et al*. 2010).

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 (Cao H *et al*. 2010).

2.1.4.1 Category 1:

Technical types: This category of cupping is in line with technique used in doing cupping. It includes dry cupping, flash cupping, wet cupping and massage cupping.

2.1.4.1.1 Dry cupping:

Dry cupping (Lauche R *et al,* 2011) is also given other names such as static cupping or retained cupping (Cao H *et al*, 2010).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 JI, 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 LM *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 pressure is higher than the negative pressure inside the cup allowing the skin to pullout (Lauche R *et al*, 2011).

Cupping is applied to increase the circulation of blood and lymph to the local area and also to relieve painful muscle tension (Lauche R *et al*, 2011).

Cupping effectively treats pain and also enhances a patient's general feeling of (Lauche R *et al,* 2011). Risk of burn, scar formation, and dermatitis are the main disadvantages of this method.

2.1.4.1.2 Flash cupping:

Flash cupping (Cao H *et al,* 2012), also referred to as empty cupping (Cao H *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 H *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 H *et al*. 2010).

2.1.4.1.3 Wet cupping:

Wet cupping (Kim JI *et al,* 2011), has been given several other names: full cupping (Al-Rubaye KQ, 2012). Blood letting cupping (Ahmed SM *et al*, 2004) and bleeding cupping. This method is used most frequently in traditional medicine (Cao H *et al,* 2010) surgical instrument is used to scrape the skin and the cup is then applied to suck blood (Ahmed SM *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 JI *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 (Cao H *et al*. 2010).

2.1.4.1.4 Massage cupping:

Massage cupping (Jiang T et al, 2004) also known as moving cupping (Cao H *et al*, 2010) dynamic cupping (Winkes MB *et al*, 2012) and gliding cupping (Tham LM *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 LM *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 (Cao H *et al*. 2010).

2.1.4.2 Category 2:

The power of suction related types: This category of cupping types is classified according to the level of negative pressure inside the cups used in doing cupping. It includes light cupping, medium cupping and strong cupping (AL-Shamma YM and Abdil Razzaq A, 2009) and pulsatile cupping therapy (Teut M *et al*, 2012).

2.1.4.2.1 Light cupping:

Practitioners produce a weak suction in the cup to do light cupping (AL-Shamma YM and Abdil Razzaq A, 2009). It is suitable for children and elderly people. The pressure inside the cup is between 100 and less than 300 millibar which is a unit of atmospheric pressure. Practitioners do one to two full manual pump suctions to perform light cupping (Tham LM *et al*, 2006).

It is a light method of cupping that can be used for elderly people and in sensitive body parts like the face. Light cupping pressure used in massage, dry and flash cupping techniques and may be used to treat pain disorders for elderly people and facial massage (Cao H *et al*. 2010).

The advantage of light cupping is that it does not leave cupping marks on most of cases. Conversely, fall of cup is the most frequently reported disadvantage of light cupping (Cao H *et al*. 2010).

2.1.4.2.2 Medium cupping:

Medium cupping (AL-Shamma YM and Abdil Razzaq A, 2009) is a medium strength, and general purpose cupping. The pressure inside the cup remains between 300 and less than 500 millibar. Practitioners do three to four full manual pump suctions to perform medium cupping (Tham LM *et al*, 2006). It is a general purpose cupping method and the negative pressure is suitable for all other types. Medium cupping pressure may be used to treat musculoskeletal pain conditions, headaches and both increase blood circulation. Frequently observed cupping marks is one of its disadvantages so, it is advisable to not use this method on face (Cao H *et al*. 2010).

2.1.4.2.3 Strong cupping:

Strong cupping is done by creating high negative pressure inside the cups (AL-Shamma YM and Abdil Razzaq A, 2009). Suction is intense and, therefore, it is not suitable for children and elderly people. The negative pressure inside the cup is above 500 millibar. Practitioners do five or more full manual pump suctions to perform strong cupping (Tham LM *et al,* 2006). The practitioners should take special care while performing strong type of cupping because they produce a high negative pressure on the skin associated with the risk of inflammation, pain and discomfort. The risks of dermatitis and skin burn are the two main disadvantages of this method (Cao H *et al*. 2010).

2.1.4.2.4 Pulsatile cupping therapy:

Pulsatile cupping is special type of cupping therapy (Teut M *et al,* 2012). The pressure inside the cups is not constant but variable. It is used in randomized clinical trials evaluating the efficacy of cupping therapy in the treatment of

osteoarthritis (Teut M *et al*, 2012). Pulsatile cupping is administered by a mechanical cupping device with flexible silicone and plastic cups according to the treatment area. The device generates a pulsatile (changeable) negative pressure inside cup. Negative pressure varies between 100 and 200 millibar, at the interval of 2 seconds (Teut M *et al*, 2012). This method is found to relieve symptoms of osteoarthritis of the knee compared to no intervention (Teut M *et al*, 2012). It is one of the new cupping types tested by randomized clinical trial (Teut M *et al*, 2012) and more research is needed using pulsatile cupping therapy (Cao H *et al*. 2010).

2.1.4.3 Category 3:

Method of suction related types: This category of cupping types is classified according to how the practitioners create negative pressure inside the cup. It includes fire cupping, manual vacuum cupping and electrical vacuum cupping (Cao H *et al*. 2010).

2.1.4.3.1Fire cupping therapy:

Fire cupping is a type of cupping done by creating negative pressure inside the cups by using fire (Huang T *et al*, 2011). It is used with glass, ceramic and bamboo cups that have no valves (a valve is a tool for controlling the passage of air through the cup). In China, the traditional cupping method is usually performed with fire; a piece of paper or cotton alighted and inserted into cup directly or a piece of cotton is soaked with 95% alcohol, applied to the end of a stick and then ignited. The burning stick is circled in the cup a few times and then removed (Huang T *et al,* 2011). The cup is applied immediately on the skin surface (Huang T *et al*, 2011). There is a risk of skin burn in this cupping type because of using fire.

2.1.4.3.2 Manual vacuum cupping therapy:

Manual cupping (Duh FC and Chiu YH, 2015) has also other names: vacuum cupping and opening cupping (Huang T *et al*, 2011). It is done by creating negative pressure inside the cups by using manual suction pump (Duh FC and Chiu YH, 2015). Self suction cups using rotator on top of cup, or squeeze rubber top can be added to this type. The main advantages of this method are: experimental studies showed that the increase in blood flow is more evident by using this type of cupping than the traditional fire one.

In addition, this cupping instrument is also a new technique in the modernization of cupping (Huang T *et al*, 2011). Reusing the manual pump without sterilization by some practitioners is the main disadvantage of this method (Cao H *et al*. 2010).

2.1.4.3.3 Electrical vacuum cupping therapy:

Electrical vacuum cupping is a type of cupping in which negative pressure is created inside the cup by using electrical suction pump or apparatus (Duh FC and Chiu YH, 2015). The advantages of this type are that the therapists can adjust the negative pressure freely, can produce a negative pressure pulse, and connect several cups (Duh FC and Chiu YH, 2015). It can be used in medical researchers to measure and adjust negative pressure inside cup (Cao H *et al*. 2010).

2.1.4.4 Category 4:

Materials inside cups related types: This category of cupping types is classified according to the material inside the cups. Some new cupping devices contain magnets, laser probe, and electrical stimulant. They used also more than one therapy in the same session by complementing the value of cupping therapy to other traditional therapies (Cao H *et al*. 2010).

It includes needle cupping, hot cupping, herbal cupping, magnetic cupping, laser cupping, electrical stimulation cupping and water cupping (Cao H *et al*. 2010).

2.1.4.4.1 Needle cupping:

Needle cupping is done by applying the acupuncture needle first, and then the cup is applied over it (Duh FC *et al,* 2015). Using small short needle and avoid abdomen and chest areas are essential to protect the patient from serious adverse events like penetrating organ, or causing pneumothorax. Skin disinfection (aseptic measures) and wearing personal protective equipment are important for practitioners (Cao H *et al*. 2010).

2.1.4.4.2Hot cupping:

Dried herb, called Moxa is used to do hot cupping (Anees S *et al,* 2015) or Moxa cupping (Cao H *et al,* 2010). Therapists use a needle, warmed by Moxa, and then the cup is applied over it. Usually, they use special technique to protect skin from burning by using a thin aluminum layer under the hot Moxa. Moxa is a dried Mugwort leaves used in Chinese medicine in a procedure called Moxibustion, a form of acupuncture. Observing patient during the procedure is very important because the patient is at risk of burn, which is the main disadvantage of this method (Cao H *et al*. 2010).

2.1.4.4.3 Herbal cupping:

Herbal cupping (Wu X *et al,* 2013). or medicinal cupping (Cao H *et al,* 2011) is done by boiling cups in a suitable herbal tincture, and then applied to the skin (Wu X *et al,* 2013). The herbs are placed into a deep pan and boiled in water for 30 minutes. Then bamboo cups are placed in the herbal tincture for 5 minutes to soak. Each cup is removed from the pan, allowed to cool briefly (about 1 minute) and then applied to a pain area (Cao H *et al,* 2011).

Cups were left for 10 minutes. One session daily and the entire treatment require 15 sessions (Cao H *et al,* 2011). Caution is taken to minimize the risk of burns or blisters from the hot cups or hot water dripping On to the skin (Cao H *et al,* 2011). Allowing cup to cool is important to protect patient from the risk of burn injury. Sharp edges of bamboo cups, the risk of burn and the difficulty in cup sterilization after use are some of the disadvantages of this method (Cao H *et al*. 2010).

2.1.4.4.4 Magnetic cupping therapy:

Magnetic cupping therapy is done by using magnetic cupping sets that contains magnets inside the cups (Chirali and Ilkay Z, 2014). Electromagnetic stimulation increases the therapeutic effectiveness of cupping, especially treatment of diseases related to joints (Chirali and Ilkay Z, 2014). The main advantage of this therapy is the dual effect on patients. The development of skin ulcers on prolonged application is the main disadvantage (Cao H *et al*. 2010).

2.1.4.4.1.5 Laser cupping therapy:

This method uses new cupping devices that contain acupuncture laser probe inside the cups. It stimulates acupuncture points by laser stimulation in addition to cupping (Lin ML *et al,* 2012).

The researchers did not evaluate and test this method properly, and hence needs research in future. The advantage of this method is the dual effect of laser acupuncture and cupping therapy. The cost of this device may be the main disadvantage until it is tested properly.

2.1.4.4.6 Electric stimulation cupping therapy:

Electrical stimulation cupping is a method of providing electric stimulation simultaneously with cupping therapy. The electrical stimulation during treatment is similar to transcutaneous electrical nerve stimulation (TENS)

stimulation. Thus the combined two therapies enhance the overall effect and used for stimulating points and muscles by electric and vacuum stimulation (Chirali and Ilkay Z, 2014). The advantage of this method is the dual effect of electrical stimulation and cupping therapy.

2.1.4.4.7 Water cupping:

Water cupping is done by using warm water inside the cup during cupping session. It involves filling a third of the cup with warm water. Whilst holding the cup close to the client with one hand, bring it close to the point to be cupped and insert burning cotton wool into the cup, then swiftly and Simultaneously turn the cup onto the skin. When performed properly, no water spillage occurs (Al-Rubaye KQ, 2012). Beneficial for treating asthma and related conditions including dry cough (Chirali and Ilkay Z, 2014). But certainly not in the acute stages of these diseases. Water spillage is the main disadvantage of this method.

2.1.4.5 Category 5:

Area treated related types: This category of cupping types is classified according to the body part considered for the cups application. This is a new concept of cupping therapy practice to specify and concentrate on certain body areas. This category includes Pedi cupping, abdominal cupping, facial cupping, female cupping and male cupping (Cao H *et al*. 2010).

2.1.4.5.1 Pedi cupping:

Pedi Cupping is a combination of reflexology, massage cupping and plantar fascial release on the leg and foot (Shaban T, 2013). This method could be used to treat musculoskeletal pain in the legs and lower compartment. This method may be used to treat plantar fasciitis as other leg ailments.

2.1.4.5.2 Abdominal cupping:

When Cupping is used for the abdomen, it is called abdominal cupping. The treatment sequence starts from the top, under the sternum and moving the cup towards the outer aspect of the body (Chirali and Ilkay Z, 2014). Circling the umbilicus clockwise and then increasing the circle. It begins with flash cupping and continues with massage cupping on abdomen (Chirali and Ilkay Z, 2014). It stretches the walls of the organs, increase blood circulation and promote the digestive system (Shaban T, 2013). This method could be used to treat digestive problems and in the management of obesity. Stimulation of blood circulation and acupuncture points which control mood and appetite are the main suggested mechanisms of action (Lacey JM *et al*, 2003).

2.1.4.5.3 Facial cupping:

Facial cupping is a rejuvenation treatment of face by cups. It is used mainly for beauty reasons. Small silicon cups are used for facial cupping. The practitioners often use special massage cupping techniques (Chirali and Ilkay Z, 2014). The benefits of facial cupping are multiple including oxygen-rich blood is forced to the face, fluid circulation is encouraged, and the lymphatic system is Activated, and all this resulting in healthier and livelier looking skin (Chirali and Ilkay Z, 2014). Some practitioners do not use the infection control measures, which is the main disadvantage of this method.

2.1.4.5.4 Female cupping:

Female cupping is also called Breast cupping therapy (Chirali and Ilkay Z, 2014) can be done by the use of special cup sizes and sets to stimulate and support female breasts (Shaban T, 2013). The cupping treatment begins with light to medium cupping (Chirali and Ilkay Z, 2014).

Two major factors influence the outcome of breast enhancement treatment: One is age, and the other is the body mass index. Patients over the age of 20 or severely underweight have a poor prognosis (Chirali and Ilkay Z, 2014).

2.1.4.5.5 Male cupping:

Male cupping is the use of vacuum erection device to stimulate and support erection function (Shaban T, 2013). The device includes a clear plastic cylinder and a vacuum pump. This method uses negative pressure to increase blood flow and, thus, helps in the treatment of erectile dysfunctions.

2.1.4.6 Category 6:

Other types: This category includes cupping types that are not classified in other categories. It includes sports cupping, cosmetic cupping and aquatic cupping.

2.1.4.6.1 Sports cupping:

Cupping is used for the treatment of sports and athletic injuries and for rehabilitation purposes. (Chirali and Ilkay 2014 and LaCross Z 2014). Myofascial decompression is an alternative term used describing this method of cupping therapy. Myofascial decompression is specific techniques have developed for cupping therapy to aid in healing of musculoskeletal pathologies. Movement patterns and functional exercise with the cups attached to specific sites (LaCross Z, 2014). One of the best examples of it is the treatment of hamstrings conditions by cupping (LaCross Z, 2014).

The treatment begins with a light scraping of the area to increase blood flow and screen for soft tissue adhesions. The cups are then applied on hamstring for 3 minutes then the athlete performs a series of active movement patterns, ten hamstring curls, and ten prone straight legs Rises with the cups in place. The therapist then passively moves the patient's leg through passive range of motion with the cups still in place (LaCross Z, 2014).

The final step is a sliding of the cups along the treatment area following a distal to proximal pattern (LaCross Z, 2014). Myofascial decompression is used as an intervention for soft tissue injuries like hamstrings strain (LaCross Z, 2014).

2.1.4.6.2 Cosmetic cupping:

Cosmetic cupping is one of the new concepts of cupping therapy that was introduced in spas and beauty salons. In cosmetic cupping, devices are used to enhance body functions and health. Selection of the right cup size is important; small size cups are used for facial cupping and large Cups are used for the arms and legs (Chirali and Ilkay Z, 2014).

2.1.4.6.3 Aquatic cupping:

Aquatic cupping is doing cupping underwater. Muscles tend to stretch much underwater and doing cupping may help in this situation. This method combines cupping therapy with aquatic therapy. Aquatic cupping is water-based treatments of therapeutic value. It is used for rehabilitation, and musculoskeletal diseases. In aquatic therapy, Hot water produces sedation effect, and cold water produces stimulation effect (Chirali and Ilkay Z, 2014).

2.1.5 Steps of wet cupping:

The steps were as follow:

1- Primary sucking: The cups were placed on the designated sites and a negative pressure was created by manual suction using the provided pump with the kit. The cups were left for a period of 5 minutes after ensuring their firm attachment to the skin (Refaat B *et al,* 2014).

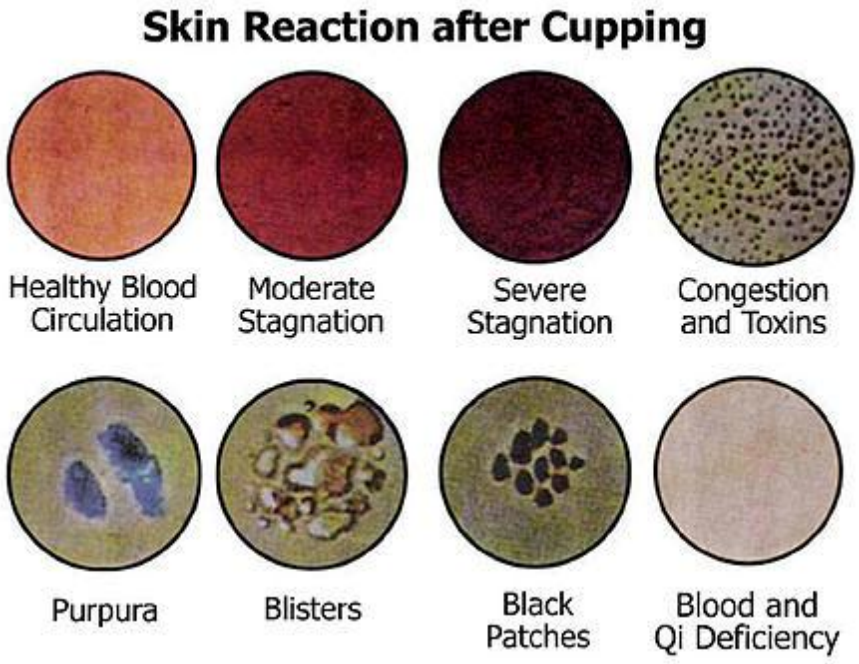
2- Incision: The cups were removed and 10-12 superficial incisions were made on each designated area of the skin using sterile surgical blades.

3- Blood-letting: After soaking the cups, they were replaced back on the designated areas of the skin and negative pressure was created as previously described. The cups were left on the skin till they were filled with blood from the capillary vessels.

4- Removal: The cups were removed approximately after 3 minutes and new cups were placed on the same areas as previously mentioned. The used cups were socked in beta dine for sterilization.

5- Blood-letting: The process of blood-letting was repeated for 3 times in total.

6- Clean and sterilize: The cupping areas were cleaned using betadine followed by placement of clean dressing (Refaat B *et al*, 2014).



2.1.6 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 (Feroz.O.L, 2013).

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)] (Feroz.O.L, 2013).

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)] (Feroz.O.L, 2013).

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)] (Feroz.O.L, 2013).

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 hence performed hijamah. [Ahmed (1/305) the Hadeeth is Hasan] (Feroz.O.L, 2013).

2.1.7 When alhijamah is done:

Anas ibn Maalik (RA) reported that the Rasul (SAW) said, "Whoever wants to perform Hijamah then let him search for the 17th, 19th and 21st…" [Saheeh Sunan ibn Maajah (3486)]. These are the generally accepted dates for Hijamah, irrespective of what day of the week they fall on, though there are

other Ahadeeth that seem to Prohibit having it done on particular days of the week, these hadeeth are categorized as Deaf however and as such the days mentioned in them Are not strictly prohibited, they are mentioned here for completeness:

Ibn Umar (RA) reported that the Rasul (SAW) said, "Hijamah on an empty stomach is best. In it is a cure and a blessing. It improves the intellect and the memory. So cup yourselves with the blessing of Allaah on Thursday. Keep away from Hijamah on Wednesday, Friday, Saturday and Sunday to be safe. Perform Hijama on Monday and Tuesday for it is the day that Allaah saved Ayoub from a trial. He was inflicted with the trial on Wednesday. You will not find leprosy except (by being cupped) on Wednesday or Wednesday night."[Sunan ibn Maajah (3487)] (Feroz.O.L, 2013).

2.1.8 Hijamah in illness:

When a patient is complaining of a particular condition, i.e. they are not in good health, but suffering from a particular illness for which Hijamah is indicated then this is termed Hijama-bil-Mardh (Hijamah in illness).

In illness the rules of Hijamah are different. For this reason Imam Ahmad ibn Hanbal would have Hijamah at any time of the month and hour of the day as a result of the need of performing Hijamah due to illness. (Feroz.O.L, 2013).

2.1.9 Phlebotomy vs. Hijamah:

Phlebotomy is often confused with Hijamah yet the two are very different in their method and effect on the body. Phlebotomy is the bleeding of veins via the use of a hypodermic needle and results in releasing of blood from the inner parts of the body as opposed to the outer part which is achieved through traditional Hijamah. It will also be regarded as part of bloodletting, but not

Hijamah, as there are significant differences in the use of these two types of bloodletting. (Feroz.O.L, 2013).

2.1.10 How much blood should be removed?

In general Hijamah between 2 to 6 “cups” of blood are removed and this will depend on the constitution of the patient, their current state of health and the change in the color of the blood during the Hijamah procedure. Feeling the pulse is the best way to determine the current state of health. In general there are three levels of strength to the pulse; these can be described as forceless, normal force and forceful. In terms of the color of blood, the range is from dark purplish to bright red. (Feroz.O.L, 2013).

2.1.11 Areas/points that should not be bled:

There are a number of areas that should not be bled for the purpose of Hijamah. Some of these lie in close proximity to arteries; others are empirically not suited to bleeding therapies. These include the following areas:

- Over the radial artery at the wrist.
- Over the axillary artery (in the armpit).
- Over the posterior tibial artery.
- Over the external iliac artery on the lower abdomen.
- Over the carotid artery in the neck.

2.1.12 Benefits of cupping:

Cupping has been used to treat many illness conditions, but is also beneficial for healthy people who want to maintain their state of wellbeing of particular mention is the benefit that cupping promotes on the circulatory system (Feroz.O.L, 2013).

Many diseases and often painful conditions are due to poor blood circulation. Cupping encourages blood flow to these regions. In dry cupping, the toxins are brought to the underlying skin; in wet cupping, the toxins are brought out of the body, onto the surface of the skin. In this case the blood, which is diverted, is replaced by healthy blood (Feroz.O.L, 2013).

In both dry and wet forms of cupping, the partial vacuum causes the tissue Below the suction cup to swell and become engorged with blood, as blood Flow to this area increases. This enhanced blood flow under the cup draws Impurities away from the nearby tissues and organs. The release of the vacuum redirects 'toxic' blood that had pooled at the site to other areas of The body, thus allowing 'fresh' blood to replace it, so restoring normal health. Localised and deep-tissue healing takes place. In addition, wet cupping provides an instant release of toxins and pressure. By doing so, cupping Encourages and supports physis in maintaining harmony within the body (Feroz.O.L, 2013). Cupping also acts separately by stimulating the body's acupuncture points. In doing so, it leads to the release of endorphins. These are natural mediators that act like opioids (substances similar to morphine), in relieving pain and counteracting stress. Another mechanism of pain relief is the stimulation of the pressure receptors (Feroz.O.L, 2013)

2.2 Lipids Chemistry:

Lipids, commonly referred to as fats, have a dual role. First, because they are composed of mostly carbon - hydrogen (CMH) bonds, they are a rich source of energy and an efficient way for the body to store excess calories. Because of their unique physical properties, lipids are also an integral part of cell membranes and, therefore, also play an important structural role in cells. The lipids transported by lipoproteins, namely triglycerides, phospholipids, cholesterol, and cholesteryl esters, are also the principal lipids found in cells and the main focus of this section (Michael L *et al.* 2010).

2.2.1Cholesterol:

Cholesterol is an unsaturated steroid alcohol containing four rings (A, B, C, and D), and it has a single CMH side chain tail similar to a fatty acid in its physical properties4 (The only hydrophilic part of cholesterol is the hydroxyl group in the A-ring. Cholesterol is, therefore, also an amphipathic lipid and is found on the surface of lipid layers along with phospholipids. Cholesterol is oriented in lipid layers so that the four rings and the side chain tail are buried in the membrane in a parallel orientation to the fatty acid acyl chains on adjacent phospholipid molecules. The polar hydroxyl group on the cholesterol A-ring faces outward, away from the lipid layer, allowing it to interact with water by noncovalent hydrogen bonding (Michael L *et al.* 2010). Cholesterol can also exist in an esterified form called cholesteryl ester, with the hydroxyl group conjugated by an ester bond to a fatty acid, in the same way as in triglycerides. In contrast to free cholesterol, there are no polar groups on cholesteryl esters, making them very hydrophobic (Michael L *et al.* 2010).

Because it is not charged, cholesteryl esters are classified as a neutral lipid and are not found on the surface of lipid layers but instead are located in the center of lipid drops and lipoproteins, along with triglycerides (Michael L *et al.* 2010). Cholesterol is almost exclusively synthesized by animals, but plants do contain other sterols similar in structure to cholesterol. Cholesterol is also unique in that, unlike other lipids, it is not readily catabolized by most cells and, therefore, does not serve as a source of fuel. Cholesterol can, however, be converted in the liver to primary bile acids, such as cholic acid and chenodeoxycholic acid, which promote fat absorption in the intestine by acting as detergents (Michael L *et al.* 2010). A small amount of cholesterol can also be converted by some tissue, such as the adrenal gland, testis, and ovary, to steroid hormones, such as glucocorticoids, mineralocorticoids, and estrogens (Michael L *et al.* 2010). Finally, a small amount of cholesterol, after first being converted to 7-dehydrocholesterol, can also be transformed to vitamin D3 in the skin by irradiation from sunlight (Michael L *et al.* 2010).

2.2.2 Cholesterol Measurement:

Early method used to measure cholesterol by using of strong acids but it's not specific a partial or full extraction of an organic solvents was sometimes used to improve specificity. The current reference method for cholesterol uses hexane extraction after hydrolysis with alcoholic KOH followed by reaction with Liebermann-Burchard color reagent, which comprises sulfuric, and acetic acids and acetic Anhydride (Michael L *et al.* 2010). This multistep manual method is complicated but gives good agreement with the gold standard method developed and applied at the U.S. National Institute for Standards and Technology, the so-called Definitive Method, using isotope dilution mass spectrometry (Michael L *et al.* 2010).

Enzymatic reagents have generally replaced strong acid chemistries in the routine laboratory. Enzymes, selected for specificity to the analyte of interest, provide reasonably accurate quantitation without the necessity for extraction or other pretreatment. Enzymatic reagents are mild compared with the earlier acid reagents and better suited for automated chemistry analyzers (Michael L *et al.* 2010).

2.2.3 Low-Density Lipoproteins:

LDL primarily contains apo B-100 and is more cholesterol rich than other apo B–containing lipoproteins .They form as a consequence of the lipolysis of VLDL. LDL is readily taken up by cells via the LDL receptor in the liver and peripheral cells (Michael L *et al.* 2010). In addition, because LDL particles are significantly smaller than VLDL particles and chylomicrons, they can infiltrate into the extracellular space of the vessel wall, where they can be oxidized and taken up by macrophages through various scavenger receptors (Kruth HS, 2002). Macrophages that take up too much lipid become filled with intracellular lipid drops and turn into foam cells (Michael L *et al.* 2010), which are the predominant cell type of fatty streaks, an early precursor of atherosclerotic plaques (Warnick GR *et al,* 2006).

LDL particles can exist in various sizes and compositions and have been separated into as many as eight subclasses through density ultracentrifugation or gradient gel electrophoresis. (Warnick GR *et al*, 2006). The LDL subclasses differ largely in their content of core lipids; the smaller particles are denser and have relatively more triglyceride than cholesteryl esters. (Carmena R *et al,* 2004). Recently, there has been great interest in measuring LDL subfractions, because small, dense, LDL particles have been shown to be more proatherogenic and may be a better marker for coronary heart disease risk (Carmena R *et al,* 2004).

2.2.4 Low-Density Lipoprotein Measurement:

LDL cholesterol, well validated as a treatable risk factor for CHD, is the primary basis for treatment decisions in the NCEP clinical guidelines.

The most common research method for LDL cholesterol quantitation and the basis for the reference method has been designated betaquantification, in which beta designation refers to the electrophoretic term for LDL. Beta-quantification combines ultracentrifugation and chemical precipitation (Michael L *et al.* 2010). Ultracentrifugation has been preferred for VLDL separation because other methods, such as precipitation, are not as specific for VLDL and may be subject to interference from chylomicrons (Michael L *et al.* 2010). In general, ultracentrifugation is a robust but tedious technique that can give reliable results provided the technique is meticulous. In a separate step, chemical precipitation is used to separate HDL from either the whole serum or the infranate obtained from ultracentrifugation. Cholesterol is quantified in serum, in the 1.006 g/mL infranate, and in the HDL supernate by enzymatic or other assay methods; LDL cholesterol is calculated as the difference between cholesterol measured in the infranate and in the HDL fraction (Michael L *et al.* 2010).

2.2.5 High-Density Lipoproteins:

HDL the smallest and most dense lipoprotein particle is synthesized by the liver and intestine (Michael L *et al.* 2010). HDL can exist as either disk-shaped particle, more commonly, spherical particles (Michael L *et al.* 2010). Discoidal HDL typically contains two molecules of apo A-I, which form a ring around a central lipid bilayer of phospholipid and cholesterol. Discoidal HDL is believed to represent nascent or newly secreted HDL and is the most active form in removing excess cholesterol from peripheral cells (Morgan J *et*

al, 2004). The ability of HDL to remove cholesterol from cells, called reverse cholesterol transport, is one of the main mechanisms proposed to explain the antiatherogenic property of HDL. When discoidal HDL has acquired additional lipid, cholesteryl esters and triglycerides form a core region between its phospholipid bilayer, which transforms discoidal HDL into spherical HDL. HDL is highly heterogeneous separable into as many as 13 or 14 different subfractions. There are two major types of spherical HDL based on density differences: HDL2 and HDL3. HDL2 particles are larger in size and richer in lipid than HDL3 and may reflect better efficiency in delivering lipids to the liver (Morgan J *et al*, 2004).

2.2.6 High-Density Lipoprotein Measurement:

HDL for many years was separated almost exclusively by chemical precipitation, involving a two-step procedure with manual pretreatment (Michael L, *et al.* 2010). A precipitation reagent added to serum or plasma aggregated non-HDL lipoproteins, which were sediment by centrifugation, HDL is then quantified as cholesterol in the supernatant, usually by one of the enzymatic assays modified for the lower HDL cholesterol range (Michael L *et al.* 2010). The earliest common precipitation method used heparin in combination with manganese. But manganese interfered with enzymatic assays so an alternative reagents were developed; Sodium phosphotungstate with magnesium became commonly used (Michael L *et al.* 2010).But because of its sensitivity to reaction conditions and greater variability it was largely replaced by dextran sulfate (a synthetic heparin) with magnesium. A significant problem with HDL precipitation methods is interference from elevated triglyceride levels, indicated by cloudiness, turbidity, or particulate matter floating in the supernate, results in over estimation of HDL cholesterol (Michael L *et al.* 2010).

High-speed centrifugation reduces the proportion of turbid supernatant. Pre dilution of the specimen promotes clearing but may lead to errors in the cholesterol analysis. Turbid supernates may also be cleared by ultrafiltration, a method that is reliable but tedious and inefficient. Because of these drawbacks and the fact that the laborious pretreatment step is not amenable to full automation, the precipitation methods became increasingly out of step with the modern automated clinical laboratory (Michael L *et al.* 2010). The result has been development of a new class of direct, sometimes termed homogeneous, methods which automate the HDL quantification, making them better suited for the modern chemistry laboratory (Michael L *et al.* 2010). Specific polymers, detergents, and even modified enzymes are used to suppress the enzymatic cholesterol reaction in lipoproteins other than HDL (Michael L, *et al.* 2010). In general; a first reagent is added to "block" non-HDL lipoproteins, followed by a second reagent with the enzymes to quantify the accessible (HDL) cholesterol. Homogeneous assays, which appear to be highly precise and reasonably accurate, have generally replaced pretreatment methods in the routine laboratory (Michael L, *et al.* 2010).

2.2.8 Lipoprotein Physiology and Metabolism:

2.2.8.1 Lipid Absorption:

Because fats are water insoluble, special mechanisms are required to facilitate the intestinal absorption of the 60 to 130 g of fat per day in a typical Western diet. During the process of digestion, pancreatic lipase, by cleaving off fatty acids, first converts dietary lipids into more polar compounds with amphipathic properties (Levy E *et al*, 2007). Thus, triglycerides are transformed into monoglycerides and diglycerides; cholesterol esters are transformed into free cholesterol; and phospholipids are transformed into lysophospholipids.

These amphipathic lipids in the intestinal lumen form large aggregates with bile acids called micelles. Lipid absorption occurs when the micelles come in contact with the microvillus membranes of the intestinal mucosal cells. Absorption of some of these lipids may occur via a passive transfer process; however, recent evidence suggests that, in some cases, it might also be facilitated by specific transporters, such as the NPC1L-1 transporter for cholesterol. (Davis HR and Veltri EP. Zetia 2007, Huff M *et al*, 2006). Short chain free fatty acids, with 10 or fewer carbon atoms, can readily pass directly into the portal circulation and are carried by albumin to the liver. The absorbed long chain fatty acids, monoglycerides, and diglycerides are reesterified in intestinal cells to form triglycerides and cholesteryl esters. The newly formed triglycerides and cholesteryl esters are then packaged into chylomicrons, along with apo B-48.Triglyceride absorption is efficient; greater than 90% of dietary triglycerides are taken up by the intestine. In contrast, only about half of the 500 mg of cholesterol in the typical diet is absorbed each day (Michael L *et al.* 2010).

Even a smaller fraction of plant sterols are absorbed by the intestine. Recently, a specific transport system, involving the ABCG5 and ABCG8 transporters, has been described that prevents excess absorption of dietary cholesterol and plant sterols (Michael L *et al.* 2010). Individuals with defective ABCG5 or ABCG8 transporters have a disease called sitosterolemia and have a predisposition for atherosclerosis because of increased cholesterol and plant sterol absorption (Michael L *et al.* 2010).

2.2.8.1.1 Exogenous Pathway:

The newly synthesized chylomicrons in the intestine are initially secreted into the lymphatic ducts and eventually enter the circulation by way of the thoracic duct (Zannis VI *et al*. 2004).

After entering the circulation, chylomicrons interact with proteoglycans, such as heparin sulfate, on the surface of capillaries in various tissues, such as skeletal muscle, heart, and adipose tissue. The proteoglycans on capillaries also promote the binding of lipoprotein lipase (LPL) (Michael L *et al.* 2010), which hydrolyzes triglycerides on chylomicrons. The free fatty acids and glycerol generated by the hydrolysis of triglycerides by LPL can then be taken up by cells and used as a source of energy (Michael L *et al.* 2010). Excess fatty acids, particularly in fat cells (adipocytes), are re-esterified into triglycerides for longterm storage in intracellular lipid drops. Hormone-sensitive lipase inside adipose cells can release free fatty acids from triglycerides in stored fat when energy sources from carbohydrates are insufficient for the body's energy needs. The hormones epinephrine and cortisol play a key role in the mobilization and hydrolysis of triglycerides from adipocytes, whereas insulin prevents lipolysis by adipocytes and promotes fat storage and glucose utilization. During lipolysis of chylomicrons there is a transfer of lipid and apolipoproteins onto HDL, and chlyomicrons are converted within a few hours after a meal into chylomicron remnant particles. Chylomicron remnants are rapidly taken up by the liver through interaction of apo E with specific remnant receptors on the surface of liver cells. Once in the liver, lysosomal enzymes break down the remnant particles to release free fatty acids, free cholesterol, and amino acids. Some cholesterol is converted to bile acids (Michael L *et al.* 2010). Both bile acids and free cholesterol are directly excreted into the bile but not all of the excreted cholesterol and bile salt exit the body. As previously described, approximately half of the excreted biliary cholesterol is reabsorbed by the intestine, with the remainder appearing in the stool, as fecal neutral steroids. In the case of bile acids, almost all of the

bile acids are reabsorbed and reused by the liver for bile production (Michael L *et al.* 2010).

2.2.8.1.2 Endogenous Pathway:

Most triglycerides in the liver that are packaged into VLDL are derived from the diet after recirculation from adipose tissue (Parhofer KG and Barrett PH, 2006). Only a small fraction is synthesized de novo in the liver from dietary carbohydrate. VLDL particles, once secreted into the circulation, undergo a lipolytic process similar to that of chylomicrons. VLDL loses core lipids causing dissociation and transfer of apolipoproteins and phospholipids to other lipoprotein particles, primarily by the action of LPL. During this process, VLDL is converted to VLDL remnants, which can be further transformed by lipolysis into LDL. About half of VLDL is eventually completely converted to LDL, and the remainder is taken up as VLDL remnants by the liver remnant receptors (Michael L *et al.* 2010).

LDL particles are the major lipoproteins responsible for the delivery of exogenous cholesterol to peripheral cells due to the efficient uptake of LDL by the LDL receptors (Michael L *et al.* 2010). Once bound to LDL receptors, they are endocytosed by cells and transported to the lysosome, where they are degraded. The triglycerides in LDL are converted by acid lipase into free fatty acids and glycerol and further metabolized by the cell for energy or are reesterified and stored in lipid drops for later use (Akopian D and Medh JD, 2006). Free cholesterol derived from degraded LDL can be used for membrane biosynthesis, and excess cholesterol is converted by acyl-CoA:cholesterol acyltransferase (ACAT) into cholesteryl esters and stored in intracellular lipid drops (Akopian D and Medh JD, 2006). The regulation of cellular cholesterol biosynthesis is, in part, coordinated by the Availability of cholesterol delivered by the LDL receptor (Michael L *et al.* 2010). Many

enzymes in the cholesterol biosynthetic pathway (e.g., HMG-CoA reductase, the main target for the cholesterol-lowering statin-type drugs) are downregulated, along with the LDL receptor, when there is excess cellular cholesterol by a complex mechanism involving both gene regulation and post-transcriptional gene regulation. Abnormalities in LDL receptor function result in elevation of LDL in the circulation and lead to hypercholesterolemia and premature atherosclerosis (Michael L *et al.* 2010). Patients who are heterozygous for a disease called familial hypercholesterolemia, with an incidence of approximately 1:500, have only approximately half the normal LDL receptors, which results in decreased hepatic uptake of LDL by the liver and increased hepatic cholesterol biosynthesis (Michael L *et al.* 2010). The LDL that accumulates in the plasma of these individuals often leads to the development of coronary heart disease by mid-adulthood in heterozygotes and even earlier for homozygotes (Michael L *et al.* 2010).

2.2.8.1.3 Reverse Cholesterol Transport Pathway:

As previously described, one of the major roles of HDL is to maintain the equilibrium of cholesterol in peripheral cells by the reverse cholesterol transport pathway (Lewis GF and Rader DJ, 2005).

HDL is believed to remove excess cholesterol from cells by multiple pathways. In the aqueous diffusion pathway (Michael L *et al.* 2010). HDL acts as a sink for the small amount of cholesterol that can diffuse away from the cells. Although cholesterol is relatively water insoluble, because it is an amphipathic lipid, it is soluble in plasma in micromolar amounts and can spontaneously dissociate from the surface of cell membranes and enter the extracellular fluid. Some free cholesterol will then bind to HDL in the extracellular space, and, once bound, it becomes trapped in lipoproteins after it is converted to cholesteryl ester by lecithin: cholesterol acyltransferase

(LCAT), (Zannis VI *et al,* 2006) which resides on HDL. HDL can then directly deliver cholesterol to the liver by the SR-BI receptor49 and, possibly, other receptors approximately half of the cholesterol on HDL is returned to the liver by the LDL receptor, (Michael L *et al.* 2010), after first being transferred from HDL to LDL by the cholesteryl ester Transfer protein (CETP) (Michael L *et al.* 2010), which connects the forward and reverse cholesterol transport pathways. Cholesterol that reaches the liver is then directly excreted into the bile or first converted to a bile acid before excretion (Michael L *et al.* 2010).

Another pathway in which HDL mediates the removal of cholesterol from cells, involves the ABCA1 transporter (Cavelier C *et al,* 2006). The ABCA1 transporter is a member of the ATP-binding cassette transporter family that pumps various ligands across the plasma membrane (Michael L *et al.* 2010). Defects in the gene for the ABCA1 transporter lead to Tangier disease, a disorder associated with low HDL and a predisposition to premature coronary heart disease (Nofer JR and Remaley, 2005). At The exact mechanism of the ABCA1 transporter is not known, but it is believed that the transporter modifies the plasma membrane by transporting a lipid, which then enables apo A-I that has dissociated from HDL to bind to the cell membrane (Vedhachalam C *et al,* 2007).

In a detergent-like extraction mechanism, apo A-I then removes excess cholesterol and phospholipid from the plasma membrane of cells to form a discoidal-shaped HDL particle (Vedhachalam C *et al,* 2007).

The newly formed HDL is then competent to accept additional cholesterol by the aqueous diffusion pathway and is eventually converted into spherical HDL by the action of LCAT. Recently, ABCG1, another ABC transporter, has been described to facilitate the efflux of cholesterol to lipid-rich spherical HDL via

a mechanism that appears to be different than the ABCA1 transporter (Wang N et al, 2004).

2.2.8 Diagnosis and Treatment of Lipid Disorders:

Diseases associated with abnormal lipid concentration are referred to as dyslipidemias. They can be caused directly by genetic abnormalities or through environmental/ lifestyle imbalances or they can develop secondarily, as a consequence of other diseases membrane (Michael L *et al.* 2010). Dyslipidemias are generally defined by the clinical characteristics of patients and the results of laboratory tests and are not necessarily defined by the specific genetic defect associated with the abnormality. Many, but not all, dyslipidemias, regardless of associated etiology, are associated with CHD, or arteriosclerosis (Michael L *et al.* 2010).

2.2.8.1 Hypercholesterolemia:

Hypercholesterolemia is the lipid abnormality most closely linked to heart disease. One form of the disease, which is associated with genetic abnormalities that predispose affected individuals to elevated cholesterol levels, is called familial hypercholesterolemia (FH). Homozygotes for FH are fortunately rare (1:1 million in the population) and can have total cholesterol concentrations as high as 800 to 1,000 mg/dL (20–26 mmol/L) (Marais AD *et al*, 2004).

These patients frequently have their first heart attack when still in their teenage years (Marais AD *et al*, 2004). Heterozygotes for the disease are seen much more frequently (1:500 in the population) because it is an autosomal codominant disorder; a defect in just one of the two copies of the LDL receptor can adversely affect lipid levels (Michael L *et al.* 2010).

Heterozygotes tend to have total cholesterol concentrations in the range of 300–600 mg/dL and, if not treated, become symptomatic for heart disease in

their 20s to 50s. Approximately 5% of patients younger than age 50 with CAD are FH heterozygotes. Other symptoms associated with FH include tendinous and tuberous xanthomas, which are cholesterol deposits under the skin, and arcus, which are cholesterol deposits in the cornea (Michael L *et al.* 2010). In both homozygotes and heterozygotes, the cholesterol elevation is primarily associated with an increase in LDL cholesterol. These individuals synthesize intracellular cholesterol normally but lack, or are deficient in, active LDL receptors. Consequently, LDL builds up in the circulation because there are insufficient receptors to bind the LDL and transfer the cholesterol into the cells. Cells, however, which require cholesterol for use in cell membrane and hormone production (Michael L *et al.* 2010), synthesize cholesterol intracellular at an increased rate to compensate for the lack of cholesterol from the receptor mediated mechanism (Michael L *et al.* 2010). In FH heterozygotes and other forms of hypercholesterolemia, reduction in the rate of internal cholesterol synthesis, by inhibition of HMG-CoA reductase with statin drugs, stimulates the production of additional LDL receptors, particularly in the liver, which removes LDL from the circulation. Homozygotes, however, do not usually benefit from this type of therapy, because they typically do not have enough functional receptors to stimulate (Marais AD *et al*, 2004).

Homozygotes can be treated by a technique called LDL pheresis, a method similar to the dialysis treatment, in which blood is periodically drawn from the patient, processed to remove LDL, and returned to the patient (Marais AD *et al*, 2004). Most individuals with elevated LDL cholesterol levels do not have FH but are still at increased risk for premature (Michael L *et al.* 2010), and should be maintained on a low-fat, low-cholesterol diet and receive statin treatment when necessary.

2.2.8.2 Hyperlipoproteinemia:

Disease states associated with abnormal serum lipids are generally caused by malfunctions in the synthesis, transport, or catabolism of lipoproteins (Michael L *et al.* 2010). Dyslipidemias can be subdivided into two major categories: hyperlipoproteinemias, which are diseases, associated with elevated lipoprotein levels, and hypolipoproteinemias, which are associated with decreased lipoprotein levels. The hyperlipoproteinemias can be subdivided into hypercholesterolemia, hypertriglyceridemia, and combined hyperlipidemia with elevations of both cholesterol and triglycerides.

2.2.8.3 Hypolipoproteinemia:

Hypolipoproteinemias, or low levels of lipoproteins, exist in two forms:

1- Hypoalphalipoproteinemia.

2- Hypobetalipoproteinemia.

Hypobetalipoproteinemia is associated with isolated low levels of LDL cholesterol but, because it is not generally associated with CHD, it is not discussed further here (Michael L *et al.* 2010)

2.2.8.4 Arteriosclerosis:

The relationship between heart disease and dyslipidemias stems from the deposition of lipids, mainly in the form of esterified cholesterol, in artery walls. This lipid deposition first results in fatty streaks, which are thin streaks of excess fat in macrophages in the sub endothelial space. Autopsy studies have shown that fatty streaks occur in almost everyone older than age 15 (Michael L *et al.* 2010).

Chapter Three

Materials and Methods

3.1 Study design:

It is an experimental, observational retrospective, quantitative, analytical and comparative study.

3.2 Study area and study period:

The study carried out in Al-Alami Center for alternative medicine, Al-Rawabih charity organization and Al-Madeina Al-Monwara clinic from January to September 2017.

3.3 Study population and sample size:

The study was covering 40 individuals randomly selected whom are come to be treated with Al-Hijamah from different age groups.

3.4 Exclusion and inclusion criteria:

Any person came to Al-Hijamah are included in the study.

3.5 Ethical consideration:

All participants on this study were informed about the nature of study; blood samples were collected after their agreement (before cupping and after 10 days of cupping.

3.6 Sample collection and processing:

Vein side was cleaned with 70% alcohol; tourniquet was tied in space before the site of collection, the needle was inserted and 2.5ml of blood sample was collected, and then applied to heparin anti co-agulant container (steps were done before and after cupping). Then immediately separated at 3.000 rpm for 5 minutes by using the centrifuge instrument, the plasma was then separated in a plain container and storage at -21^0 C for one month.

3.7 Statistical analysis:

IBM SPSS statistic version 21, soft ware program; was applied by using Paired sample T-test and correlation relationships.

3.8 Quality control:

The precision and accuracy of all methods used in this study were choked by commercially prepared control sera before the application of the test measured.

3.9 Methods of the tests:

3.9.1Choleterol method:

Principle:

Cholesterol found in serum as cholesterol esters and free cholesterol. The cholesterol esters are hydrolyzed by cholesterol esterase and the cholesterol are then measured by oxidizing with cholesterol oxidase to form hydrogen peroxide in turn reacts with phenol and 4-aminoantipyrine present to form the quinoneimine dye. The intensity of the color is directly proportional to the level of cholesterol present in sample.

For other details see appendix no (6).

3.9.2 HDL methods:

Principle:

Low density lipoproteins are precipitated by the addition of phosphotungstic acid in the presence of magnesium ions. The HDL fraction remains in the supernatant and this is determined by cholesterol assay.

For other details see appendix no (7).

3.9.3 LDL methods:

Principle:

Low density lipoproteins are precipitated by the addition of heparin at their isoelectric point (5.04). The HDL and VLDL remain in the supernatant and can be determined by enzymatic methods.

For other details see appendix no (8).

3.9.4 Blood pressure:

Blood pressure are measured twice time pre and post cupping by using of an automatically measurement.

Chapter Four

Results

Figure (4.1): shows the frequencies of the diseases:

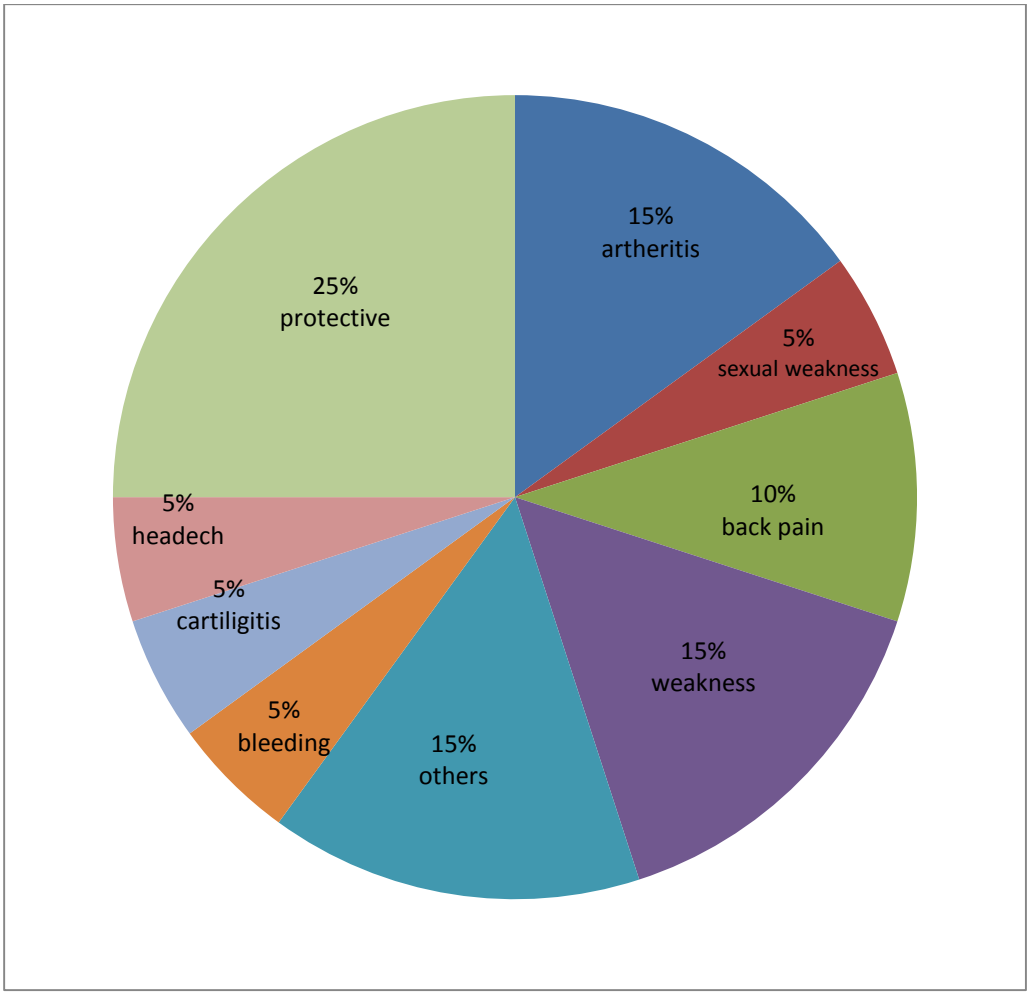


Table (4.1): Comparison between Total Cholesterol, HDL-c, LDL-c and blood pressure before and after 10 days of cupping:

Factor	Time	Number	Mean	SD	P.value
Cholesterol mg/dl	Pre	40	177	44.5	0.002
	post		161	37.3	
HDL mg/dl	pre	40	46	18.1	0.027
	post		53	22.7	
LDL mg/dl	pre	40	113	44.2	0.001
	post		93	41.2	
Systolic of BP	pre	40	122	27.7	0.04
	post		118	24.6	
Diastolic of BP	pre	40	76	16.6	0.037
	post		73	15.0	
Mean of BP	pre	40	99	21.1	0.008
	post		95	19.1	

P.value significant at 0.05.

4.3 Effect of cupping therapy on blood pressure:

There was a significant decrease in systolic blood pressure, diastolic blood pressure and even in the mean of blood pressure before Cupping compared to after cupping ; p.values are (0.04, 0.037 and 0.008) respectively.

Also there are strong correlation between the mean of blood pressure, systolic and diastolic (pre and post cupping); r.values (0.907^{**}, 0.860^{**} and 0.845^{**}) respectively.

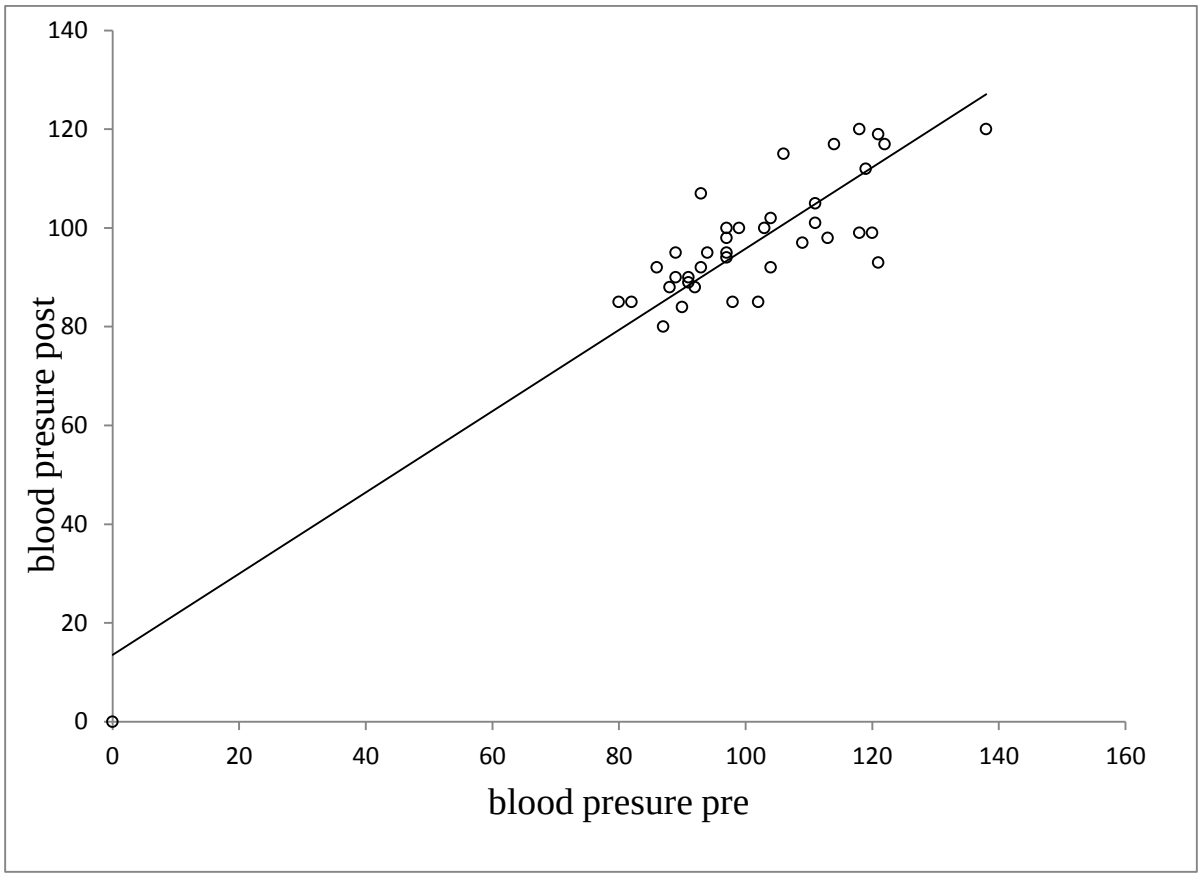


Figure NO (4.2) shows the correlation between the means of blood pressure pre and post cupping therapy.

r.value: 0.907** p.value: 0.000

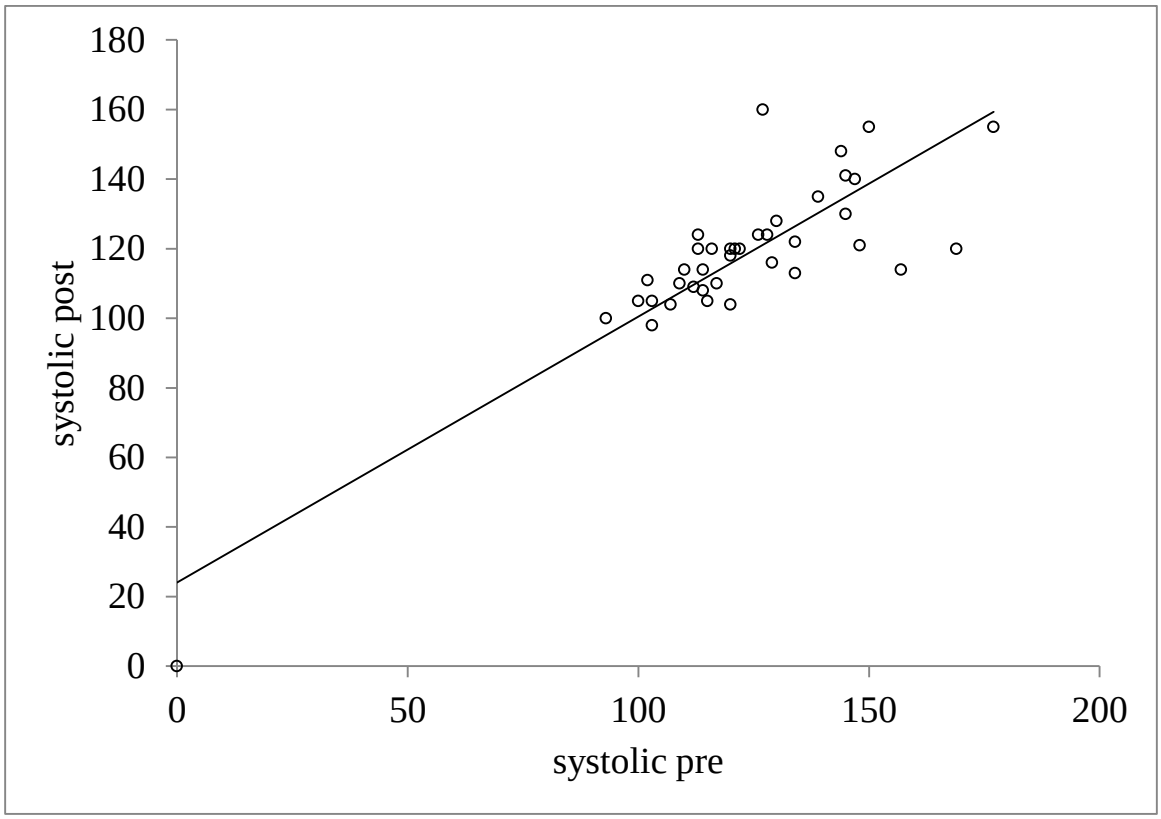


Figure NO (4.3) shows the correlation between systolic pre and post cupping therapy.

r.value: 0.860** p.value: 0.000

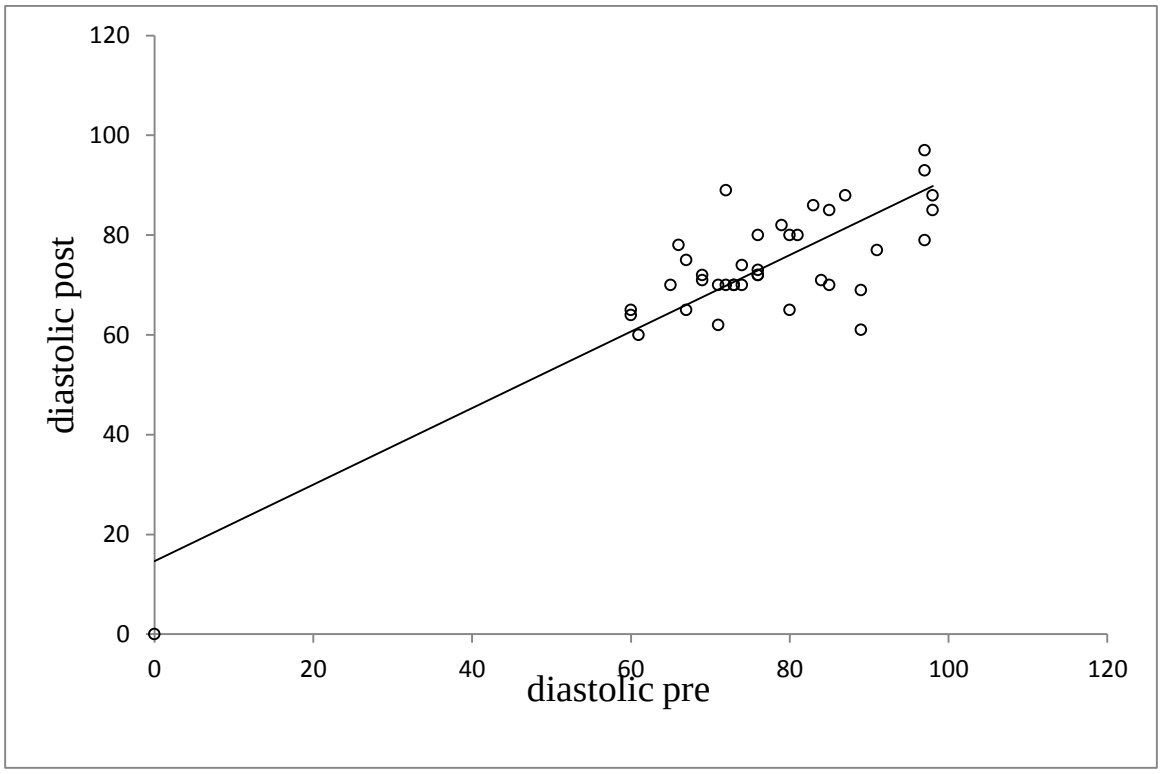


Figure NO (4.4) shows the correlation between diastolic pre and post cupping therapy**.**

r.value: 0.845** p.value: 0.000

4.4 Effect of cupping therapy on Total Cholesterol, HDL-c and LDL-c:

There was a significant decrease in total Cholesterol and LDL-c before cupping compared to after cupping; p.values are (0.002, and 0.001) respectively. How ever there was a significant increase in HDL-c before cupping compared to after cupping p.value (0.027). There are moderate correlation between total Cholesterol, LDL-c and HDL-c (pre and post cupping); r.values (0.750^{**}, 0.637^{**} and 0.631^{**}) respectively.

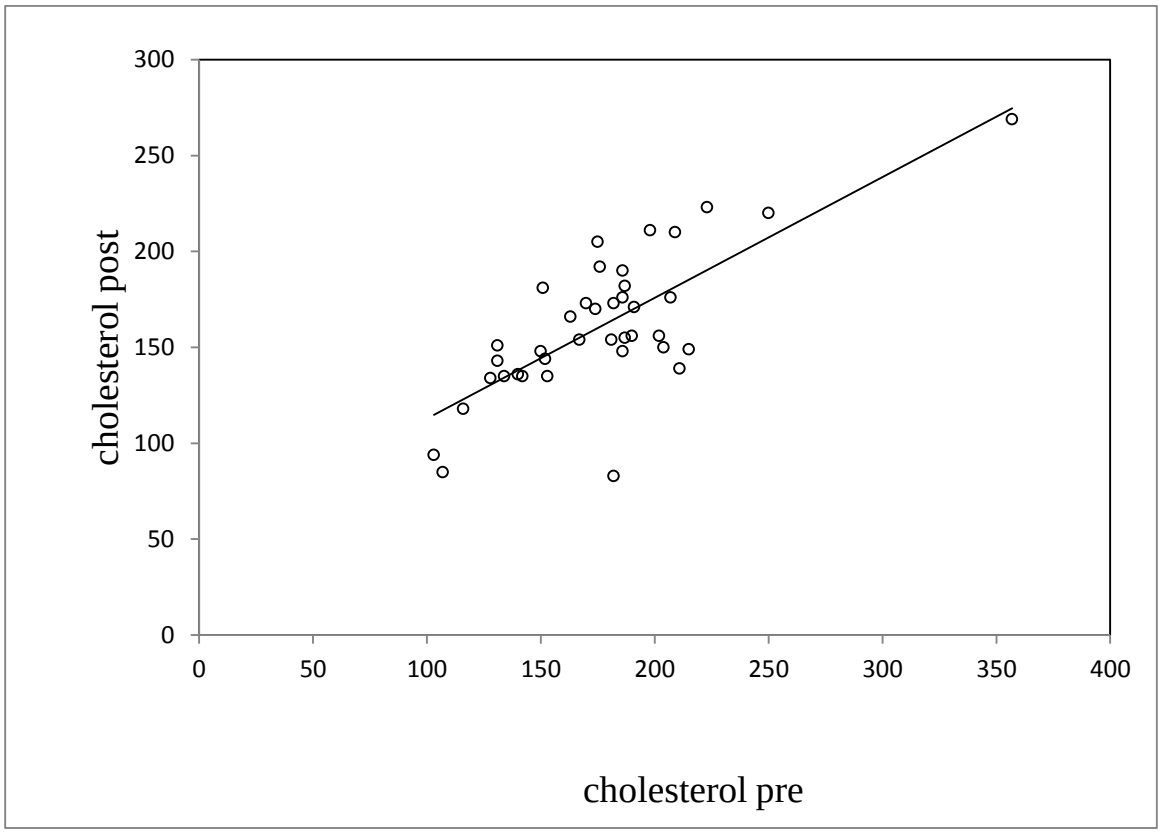


Figure NO (4.5) shows the correlation between total Cholesterol pre and post cupping therapy**.**

r.value: 0.750 ** p.value: 0.000

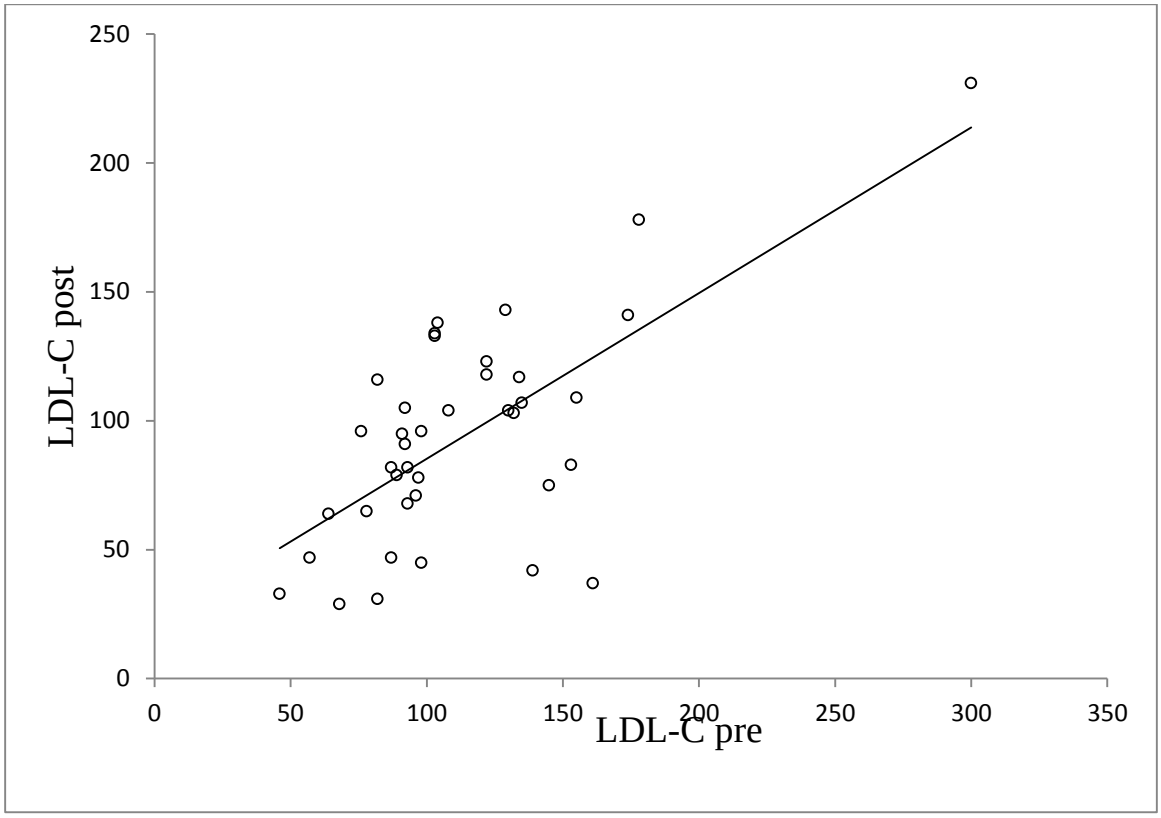


Figure NO (4.6) shows the correlation between LDL-C pre and post of cupping therapy**.**

r.value: 0.637** p.value: 0.000

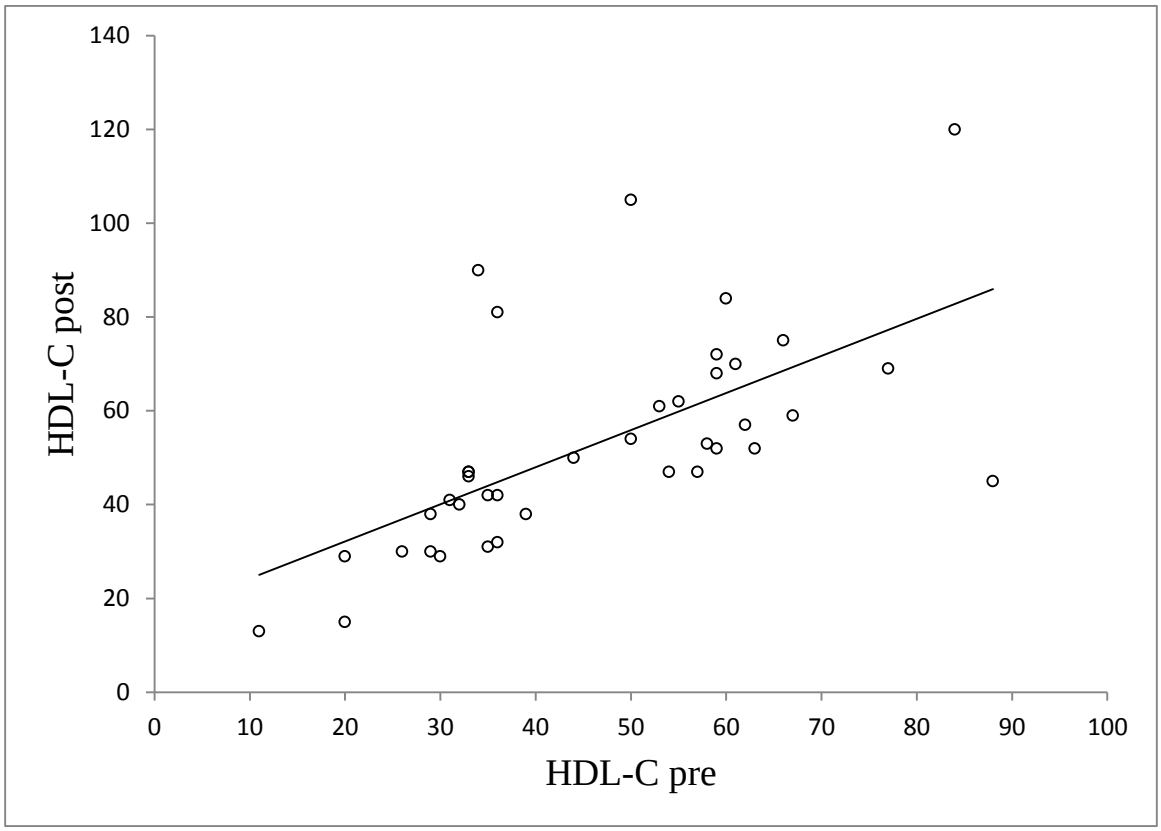


Figure NO (4.7) shows the correlation between HDL-c pre and post cupping therapy**.**

r.value: 0.631** p.value: 0.000

Chapter Five

Discussion, Conclusion and Recommendations

5.1 Discussion:

Both bleeding and cupping belong to the oldest medical procedures therapy is unlimited and the secrets of its mechanisms still mysterious. Wet cupping is part of the traditional medicine in the Arab and Islamic world as 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" (Mahmoud HS *et al* 2013, El Sayed SM *et al*, 2013). The current study aimed to evaluate the effects of cupping therapy on BP, Cholesterol, HDL-c and LDL-c blood levels.

This study showed the frequencies of the disease are; hijamah for protective 25%, arthritis15%, weakness 15%, cartiligitis 5%, bleeding 5%, sexual weakness 5%, headache 5% and other diseases 15%. Also it found that there was a significant decrease in diastolic BP, systolic BP even the mean of Blood Pressure. In addition it found a significant decrease in total Cholesterol and LDL-c levels; but a significant increase in HDL-c level.

The study found that total Cholesterol pre cupping therapy decreased compared to the post of cupping (177± 44.5 to 161 ± 37.3) mg/dl; p.value (0.002), in addition to the decreased of LDL cholesterol level after 10 days of cupping (113 ± 44.2 to 93 ± 41.2) mg/dl; p.value (0.001). However there was significant increase in HDL cholesterol level post cupping compared to the pre cupping (46 ± 18.1 to 53 ± 22.7) mg/dl. The result is in agreement with previous study which done by Fairouz K. Alshowfi, (2010) who reported that there was significant decrease in serum total cholesterol (p<0.01) and low

density lipoproteins (LDL-C) (p<0.05) and an enhanced serum high density lipoprotein (HDL-C) total cholesterol levels (p<0.05).

Also from this study found that the mean of blood pressure, systolic blood pressure and even diastolic blood pressure are decreased after 10 days of blood cupping is done; (122 ± 27.7 to 118 ± 24.6, p.value 0.04), (76 ± 16.6 to 73 ± 15.0, p.value 0. 37) and (99 ± 21.1 to 95 ± 19.1, p.value 0.008) respectively; this result is in agreement in systolic and diastolic pressure to previous study which done by Fairouz K. Alshowfi, (2010), who reported that ; there was significant difference in the reduction of blood pressure 10 days after blood cupping compared with the baseline in both systolic (p<0.05) and diastolic (p<0.01).

Also current results are agreement again with other study done by Bassem Refaat, et al (2014) in the result of HDL and LDL but disagreement in total cholesterol result; those are reported; there was a significant decrease in LDL and significant increase in HDL observed in samples collected 48 hours of the second treatment. However, there was no significant change in the levels of total cholesterol.

Finally this study found there are a moderate positive correlations between pre and post cupping samples of each total cholesterol, HDL- c and LDL - c levels r.values (0.750^{**}, 0.631^{**} and 0.637^{**}) respectively. And also found strong correlation between the mean of blood pressure, systolic and diastolic (pre and post cupping); revalues (0.907^{**}, $0.860^{**}$and $0.845^{**)}$ respectively.

5.2 Conclusion:

This study conclude that the levels of total cholesterol, LDL-c and blood pressure were decrease while the level of HDL-c was increased; these data suggest that blood cupping is a technique might be associated with decreased risk of cardio-vascular disease, obesity diseases and also helps to reduce hypertension problems.

5.3 Recommendations:

1- I encourage people to do Al-hijamah because it effective in different types of illness as Abu Hurairah (RA) narrates that Rasulullaah (SAW) said: "Jibra'eel conveyed to me that the best amongst the things that mankind uses for treatment is hijamah" (Sahih Al-Jaami 213).

2- Increase sample size and it's better to do in hypertensive volunteer and hyperlipidimic one.

References:

- **Abele J Das Schröpfen** (1996) Eine bewährte alternative Heilmethode Taschenbuch: Urban & Fischer, Mchn.
- **Ahmed SM** (2004). Madbouly NH, Maklad SS, Abu-Shady EA. Immunomodulatory effects of bloodletting cupping therapy in patients with rheumatoid arthritis. *The Egyptian Journal of Immunology/Egyptian Association of Immunologists*; 12 (2):39-51.
- **Akopian D, Medh JD** (2006). Genetics and molecular biology: *macrophage ACAT depletion—mechanisms of atherogenesis*. Cur Opin Lipidol;17:85–88.
- **AL- Shamma YM**, Abdil Razzaq A (2009) Al-Hijamah Cupping Therapy. Kufa Med J 12 (1).
- **Al-Bukhari MI** (1996). The English Translation of Sahih Al Bukhari With the Arabic Text. Translated by Muhammad Muhsin Khan, Al-Saadawi Publ.
- **Al-Rubaye KQ** (2012)**.** *The clinical and histological skin changes after the cupping therapy (Al- Hijamah).* J Turk Acad Dermatol; 6 (1):1261a1.
- **Anees S, Arafath Y**, Naaz A, Khan MQ (2015). Hijamah (Cupping therapy) as *a preventive medicine-a retro-prospective analytical study*. International Journal of AYUSH; 4 (2):88-100.
- Baguanfa treatments - Cupping therapy – ICTA; 2005. Available:www.cuppingtherapy.org/pages/ applications.html

- **Cao H,** Han M, Li X, Dong S, Shang Y, Wang Q, Xu S, Liu J. (2010). Clinical research evidence of cupping therapy in China: *A systematic literature review. BMC Complementary and Alternative Medicine*; 10 (1):70.
- **Cao H,** Hu H, Colagiuri B, Liu J (2011). Medicinal cupping therapy in 30 patients with fibromyalgia: *A case series observation. Forschende Komplementärmedizin/ Research in Complementary Medicine*; 18 (3):122-6.
- **Cao H,** Li X, Liu J (2012) *an updated reviews of the efficacy of cupping therapy.* PLoS One 7: e31793.
- **Cao H,** Liu J, Lewith GT (2010) Traditional Chinese Medicine for treatment of fibromyalgia: *a systematic review of randomized controlled trials.* J Altern Complement Med 16: 397-409.
- **Cao H**, Zhu C, Liu J (2010). *Wet cupping therapy for treatment of herpes zoster*: A systematic review of randomized controlled trials. Alternative Therapies in Health and Medicine; 16 (6):48.
- **Carmena R,** Duriez P, Fruchart JC (2004). *Atherogenic lipoprotein particles in atherosclerosis*. Circulation; 109 (Suppl III):III2–III7.
- **Cavelier C**, Lorenzi I, Rohrer L, et al (2006). *Lipid efflux by the ATPbinding cassette transporters* ABCA1 and ABCG1. Biochim Biophys Acta; 1761:655–666.
- **Chirali IZ** (1999). The cupping procedure. *InTraditional Chinese Medicine Cupping Therapy.* London: Churchill Livingstone 73-86.
- **Chirali, Ilkay Z** (2014) Elsevier Health Sciences; *Traditional Chinese medicine cupping therapy.* 11,14,127,129.

- **Christopoulou-Aletra H,** Papavramidou N (2008) Cupping: *an alternative surgical procedure used by Hippocratic physicians.* J Altern Complement Med 14: 899-902.
- **Curtis, P.** (1981). Family Practice History: *Bloodletting*. Canadian Family Physician, 27, 1030.
- **Davis HR**, Veltri EP. Zetia (2007). Inhibition of Niemann-Pick C1 like 1 (NPC1L1) to reduce intestinal cholesterol absorption and treat hyperlipidemia. J Atheroscler Thromb; 14:99–108.
- **Duh FC**, Chiu YH (2015). Vacuum cupping under various negative pressures: An experimental investigation. Vacuum; 2(7).
- **Duh FC**, Yu ZY, Chen CH (2015). *A study on avoiding burn injuries when treating patients with needle cupping*. J. Multidiscip. Eng. Sci. Technol; 2(6): 1484-9.
- **Eisenberg DM**, Davis RB, Ettner SL, Appel S, Wilkey S, et al. (1998) *Trends in alternative medicine use in the United States,* 1990-1997: results of a follow-up national survey. JAMA 280: 1569-1575.
- **El Sayed SM**, Mahmoud HS, Nabo MMH (2013) Methods of Wet Cupping Therapy (Al-Hijamah): In Light of Modern Medicine and Prophetic Medicine. Altern Integ Med 2:1-16.
- **Alshawafi** .**K**. **Fairouz** Ph.D (2010*). Effect of Blood Cupping on Some Biochemical Parameter* Med. J. Cairo Univ., Vol. 78, No. 1, June: 311-315.
- **Feroz Osman-Lat** (2013). *Islamic Cupping& Hijamah*: ISBN-10: 0991145518, ISBN-13: 978-0-9911455-1-5.
- **Huang T**, Zhang W, Huang X, Tian Y, Wang G, Zhang Y, Wang L, Litscher G (2011). *Comparing the efficacy of traditional firecupping*

and high-tech vacuum-cupping using laser doppler imaging at an acupuncture clinic in Beijing. Medical Acupuncture.; 23(1):13-8.

- **Huff MW,** Pollex RL, Hegele RA. NPC1L1 (2006). *Evolution from pharmacological target to physiological sterol transporter*. ArteriosclerThromb Vasc Biol; 26:2433–2438.
- **Jiang T**, Qi XZ, Wu B (2004). *Therapeutic effect of massage cupping ultrahort and acupuncture on cure facial-neuritis*. Health J. Anhui Tech. Coll; 3 (5):46-7.
- **Kemper KJ**, Sarah R, Silver-Highfield E, Xiarhos E, Barnes L, et al. (2000) On pins and needles? *Pediatric pain patients' experience with acupuncture.* Pediatrics 105: 941-947.
- **Kim TH**, Basargard L, Kim JI, Lee MS (2011). *Mongolian traditional style blood-letting therapy*: a brief introduction. Complement Ther Clin Pract 17: 179-183.
- **Kruth HS.** (2002).Sequestration of aggregated low-density lipoproteins by macrophages. Curr Opin Lipidol; 13:483–488.
- **Kulkarni KR (**2006). *Cholesterol profile measurement by vertical auto profile method*. Clin Lab Med; 26:787–802.
- **Lacey JM,** Tershakovec AM, Foster GD (2003). *Acupuncture for the treatment of obesity: Areview of the evidence*. International Journal of Obesity; 27(4):419-27.
- **LaCross Z (**2014**).** Treatment outcomes of myofascial decompression on hamstring pathology (Doctoral dissertation, Oklahoma State University);

- **Lauche R**, Cramer H, Choi KE, Rampp T,Saha FJ, Dobos GJ, Musial F(2011). The influence of a series of five dry cupping treatments on pain and mechanical thresholds in patients with chronic nonspecific neck pain- a randomized controlled pilot study. BMC *Complementary and Alternative Medicine*; 11(1): 1.
- **Levy E**, Spahis S, Sinnett D, et al. Intestinal cholesterol transport proteins: an update and beyond. Curr Opin Lipidol 2007; 18:310–318.
- **Lewis GF**, Rader DJ (2005). *New insights into the regulation of HDL metabolism and reverse cholesterol transport*. Circ Res; 96:1221–1232.
- **Lin ML**, Wu HC, Hsieh YH, Su CT, Shih YS, Lin CW, Wu JH. Evaluation of the effect of laser acupuncture and cupping with ryodoraku and visual analog scale on low back pain. Evidence-Based Complementary and Alternative Medicine; 2012.
- **Loukas M,** Saad Y, Tubbs RS, Shoja MM (2010) the heart and cardiovascular system in the Qur'an and Hadeeth. Int J Cardiol 140: 19-23.
- **Mahmoud HS**, Abou-El-Naga M, Omar NAA, El-Ghazzawy HA, Fathy YM, et al. (2013) Anatomical Sites for Practicing Wet Cupping Therapy (Al- Hijamah): In Light of Modern Medicine and Prophetic Medicine. Altern Integ Med 2: 1374- 1378.
- **Manz Hedwig**. (2009). the Art of Cupping, pp. 3-4. Thieme, Stuttgart, Germany Med 2006; 26:803–846.
- **Marais AD**, Firth JC, Blom DJ (2004). Homozygous familial hypercholesterolemia and its management. Semin Vasc Med; 4:43–50.

- **McMullin MF**, Bareford D, Campbell P, Green AR, Harrison C, et al. (2005) Guidelines for the diagnosis, investigation and management of polycythaemia/ erythrocytosis. Br J Haematol 130: 174-195.
- **Michael L.** Bishop, Edward P. Fody, Larry E. Schoeff. (2010). *Clinical chemistry: techniques, principles, correlations*, 6th ed. China.329-348.
- **Michalsen A**, Bock S, Lüdtke R, Rampp T, Baecker M, et al. (2009) *Effects of traditional cupping therapy in patients with carpal tunnel syndrome*: a randomized controlled trial. J Pain 10: 601-608.
- **Morgan J,** Carey C, Lincoff A, et al (2004). *High-density lipoprotein subfractions and risk of coronary artery disease*. Curr Atheroscler Rep; 6:359–365.
- **Nielsen A**. (1995). Gua Sha: A traditional technique for modern practice: Churchill Livingstone. Pepys, M. B., & Hirschfield, G. M. (2003). C-reactive protein: a critical update. Journal of Clinical Investigation, 111(12), 1805-1812
- **Nofer JR,** Remaley AT. Tangier disease: still more questions than a nswers. Cell Mol Life Sci 2005; 62:2150–2160.
- Opin Lipidol; 17:85–88.
- **Parhofer KG**, Barrett PH (2006). Thematic review series: patient-orientedresearch. What we have learned about VLDL and LDL metabolismfrom human kinetics studies. J Lipid Res; 47:1620–1630.

- **Refaat B**, El-Shemi AG, Ebid AA, Ashshi A, BaSalamah MA (2014) *Islamic Wet Cupping and Risk Factors of Cardiovascular Diseases:*

Effects on Blood Pressure, Metabolic Profile and Serum Electrolytes in Healthy Young Adult Men. Altern Integ Med 3: 151. doi:10.4172/2327- 5162.1000151

- **Reflections**, C. S. (2004). Blood-Letting *Heart Views*, 5(2), 74-85.
- **Salomonsen LJ**, Skovgaard L, la Cour S, Nyborg L, Launsø L, et al. (2011) *Use of complementary and alternative medicine at Norwegian and Danish hospitals*. BMC Complement Altern Med 11: 4.
- **Shaban T**. Cupping therapy Encyclopedia – Createspace publishing - ISBN-13: 978- 1494780517. 2013; 47-55.
- **Shaheed Abd Hameed Omar** (2009) Al-hijamah (cupping therapy): Sunnah and therapy (in Arabic). Dar Ommah for publication, Jeddah.
- **Soyuncu S**. (2009). Back pain in adult: what's your diagnosis? *European Journal of Emergency Medicine, 16(1), 57.*
- **Teut M**, Kaiser S, Ortiz M, Roll S, Binting S, Willich SN, Brinkhaus B (2012). Pulsatile dry cupping in patients with osteoarthritis of the knee– a randomized controlled exploratory trial. BMC Complementary and Alternative Medicine; 12(1):184.
- **Tham LM**, Lee HP, Lu C (2006). *Cupping from a biomechanical perspective.* Journal of Biomechanics; 39(12):2183-93.
- **Turk JL**, Allen E (1983) Bleeding and cupping. Ann R Coll Surg Engl 65: 128- 131.

- **Vedhachalam C**, Duong PT, Nickel M, et al (2007). Mechanism of ATPbinding cassette transporter A1-mediated cellular lipid efflux to

apolipoprotein A-I and formation of high density lipoprotein particles. J Biol Chem; 282:25123–25130.

- **Wang N,** Lan D, Chen W, et al (2004). A*TP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins*. Proc Natl Acad Sci U S A; 101:9774–9779.
- **Warnick GR**, McNamara JR, Boggess CN, et al (2006). Polyacrylamide gradient gel electrophoresis of lipoprotein subclasses. Clin Lab Med; 26:803–846.
- **Winkes MB**, Hoogeveen AR, Houterman S, Giesberts A, Wijn PF, Scheltinga MR (2012). Compartment pressure curves predict surgical outcome in chronic deep posterior compartment syndrome. The American Journal of Sports Medicine; 40(8):1899-905. 0363546512449324.
- **Wu X,** Hu H, Guo L, Wang H (2013). Clinical observation of post-herpetic neuralgia treated with TCM herbal cupping therapy. Zhongguo Zhen Jiu= Chinese Acupuncture & Moxibustion; 33(2):141-4.
- **Zannis VI**, Chroni A, Krieger M. Role of apoA-I, ABCA1, LCAT, and SR-BI in the biogenesis of HDL. J Mol Med 2006; 84:276–294.
- **Zannis VI,** Chroni A, Kypreos KE, et al (2004). Probing the pathways ofchylomicron and HDL metabolism using adenovirus-mediated gene transfer. Curr Opin Lipidol; 15:151–166.

Appendix (2)

Sudan University of Science and Technology

College of Graduate Studies

Study about the effect of blood cupping on total Cholesterol, HDL-c and LDL-c.

Name: ……

Patient NO ( ) Phone number: ……

Age:……years Weight: ……Kg Height: ……Cm

BMI: …… Blood pressure pre: …… Blood pressure post: ……..

Reason of hijama :……

Presence of chronic diseases:

Yes ( ) No ( )

If yes; type of disease:……...

Duration of disease;……..

Treatment:……

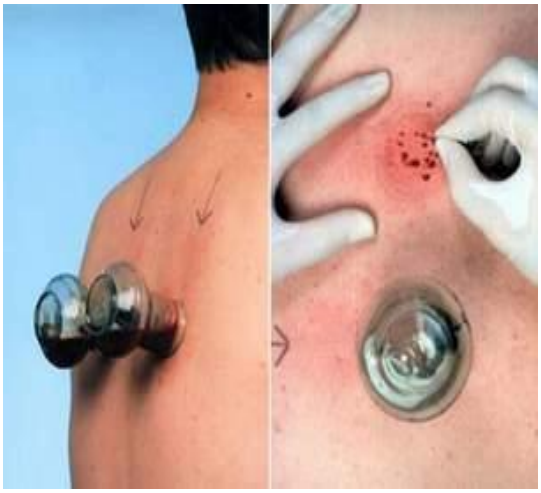
Investigation	**Level Pre Cupping**	**Level Post Cupping**
Total Cholesterol mg/dl		
HDL mg/dl		
LDL mg/dl		

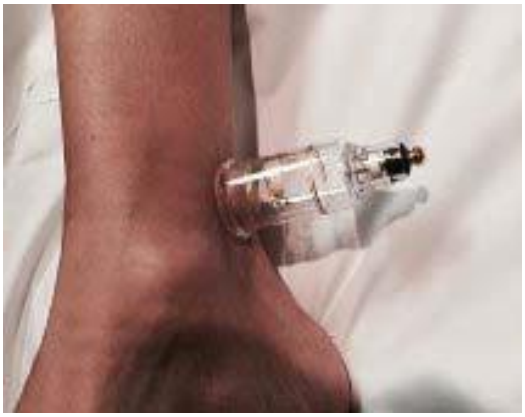
Appendix (3) :Tools of cupping therapy:

Appendix (4): Types of cupping:

1. Dry Cupping Hijamah Jaafa

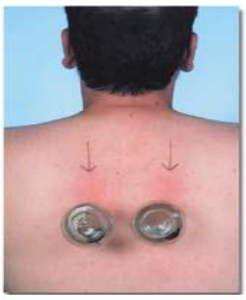
2. Wet Cupping Hijamah



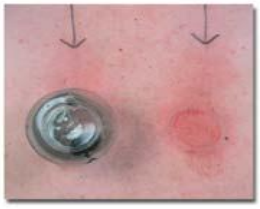


Appendix (5):

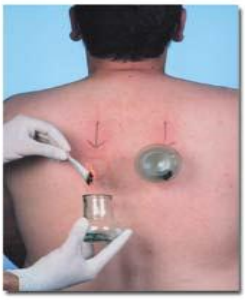
Steps of cupping:



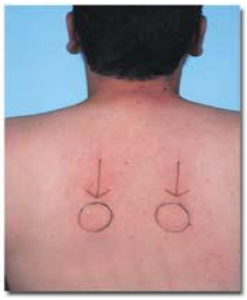
٤- وضع كؤوس الحجامة
في منطقة الكاهل لإحداث الاحتقان



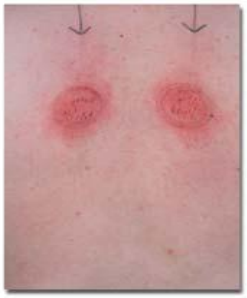
٣- منطقة الاحتقان الجلدي
التي احدثها كأس الحجامة



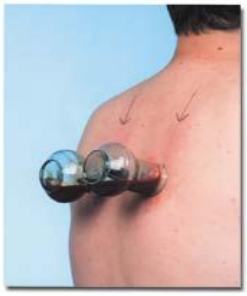
٢- مرحلة وضع كأس الحجامة
لإحداث الاحتقان الدموي



١- رسم تخطيطي يحدد منطقة
الحجامة على الكاهل



٧-التشطيبات الجراحية البسيطة
بعد إنتهاء عملية سحب الدم الفاسد



٦- سحب الدم الهرم
بواسطة كأسي الحجامة

٥- إجراء التشطيبات الجراحية السطحية
لإخراج الدم المحتقن الهرم

Appendix No 6

fortress diagnostics

CHOLESTEROL

PRODUCT CODE: BXC0261

CE QUALITY MANAGEMENT SYSTEM ISO 13485 CERTIFIED COMPANY

BXC0261A	BXC0261B	BXC0261C	BXC0261Q
2x60ml	6x60ml	8x250ml	7x50ml

STORE AT 2-8°C

INSTRUCTIONS FOR USE

FOR IN-VITRO DIAGNOSTIC USE ONLY

IVD For In Vitro Diagnostics Use Only
LOT Lot Number
REF Catalogue Number
Storage Temperature
Expiry Date (Year / Month)
Warning, Read Enclosed Documents
Instructions For Use
Manufactured By

CHOLESTEROL

CHOD-PAP (LIQUID STABLE)

Kit Contents:	BXC0261A	BXC0261B	BXC0261C	BXC0261Q
R1 Chol Reagent R4 Standard	2 x 60ml 1 x 5ml	6 x 60ml 1 x 5ml	8 x 250ml 1 x 5ml	2 x 50ml 1 x 5ml

Test Principle:
Cholesterol is present in serum as cholesterol esters and free cholesterol. The Cholesterol esters present in serum are hydrolysed by cholesterol esterase and the cholesterol is then measured by oxidizing with cholesterol oxidase to form hydrogen peroxide. The hydrogen peroxide in turn reacts with phenol and 4-aminoantipyrine present to form the red quinoneimine dye. The intensity of the dye formed is directly proportional to the level of cholesterol present in the sample.

Clinical Significance:
Elevated levels of cholesterol are primarily considered as an indication of increased risk of cardiovascular disease and should be taken into consideration combined with the overall lipid profile.

Reagent Concentration:

Chol Reagent	Pipes Buffer	50 mmol/l
	Cholesterol Oxidase	> 100 U/l
	Cholesterol Esterase	> 150 U/l
	4-aminoantipyrine	0.3 mmol/l
	Peroxidase	> 800 U/l
	Phenol	≤ 0 mmol/l
Standard	Cholesterol	200 mg/dl

Reagent Handling and Preparation:
The reagent is supplied ready to use. It is stable for up to the expiry date when kept at 2-8°C and protected from light. The reagent may develop a slight pink colouration. This will not affect performance providing the OD remains <0.300 when measured against a water blank at 505nm.

Sample:
Serum/Heparinised Plasma

EDTA plasma may also be used but tends to give lower results.
Specimen:
Do not use citrate, oxalate or fluoride.
Fasting and non-fasting samples can be used.
Centrifuge samples containing precipitate before performing assay

Stability:	5-7 days at +2 to +8°C
	3 months at -20°C

Manual Procedure:

Wavelength	Temperature	Cuvette	Measurement
500 nm, Hg 546nm	20-25°C, 37°C	1 cm light path	Against Reagent blank

Pipette into test tubes as follows:		
	Blank	**Standard / Sample**
DDH_2O	10 µl	—
Standard / Sample	—	10 µl
Chol Reagent (R1)	1000 µl	1000 µl

A serum based calibrator or an aqueous cholesterol standard can be used to calibrate this assay.
Mix well and incubate for 10 minutes at 20-25°C or 5 minutes at 37°C, then read the absorbance of the sample or standard against the reagent blank.
The endpoint is stable for 60 minutes

Calculation:
Cholesterol conc in sample= $\frac{\Delta abs\ Sample}{\Delta abs\ standard}$ x conc. of standard

Linearity:
The test is linear up to a cholesterol concentration of 20 mmol/l (774 mg/dl). Dilute samples above this concentration with 0.9% NaCl and reassay, multiplying the result by the dilution factor.

Sensitivity:
Cholesterol levels of 0.20 mmol/l (7.74 mg/dl) can be measured accurately by this method.

Precision:

	Intra Assay – Within run	
Cholesterol conc (mmol/l)	n	CV%
4.50	20	1.69 %
5.18	20	1.69 %

	Inter Assay – Between Run	
Cholesterol conc (mmol/l)	n	CV%
4.50	10	2.9 %
5.18	10	2.2 %

These characteristics were determined using an AU600 analyser. Results will vary depending on the system in use.

Limitations - Interference:
Haemoglobin values up to 200mg/dl & bilirubin values up to 5mg/dl do not interfere with the test.

Normal Values:

Cholesterol Level	Clinical Interpretation
< 5.2 mmol/l (200 mg/dl)	Normal
5.2-6.2 mmol/l (200-239 mg/dl)	Borderline Risk
> 6.2 mmol/l (240mg/dl)	High Cholesterol

These values are intended only as a guideline. Cholesterol levels can vary seasonally, according to geographic location and with time of sampling. At least 2 measurements should be made on separate occasions and the result should be taken in conjunction with other clinical and laboratory information.

Use on Automated Analysers:
This reagent is suitable for use on a range of automated analysers. Specific instructions for these applications are available on request from our technical department.
For automated use we recommend a serum based calibrator to eliminate any matrix bias which may be observed with the aqueous standard.
Fortress Calibration Serum cat. No [illegible]

Quality Control:
It is recommended that a laboratory uses normal and elevated reference control sera to verify the performance of the procedure, both performance of the reagent and any instrumentation employed in the determination. Results obtained should fall within the specified ranges.

Fortress Normal Routine Assayed Control Cat No [illegible] (10x5ml)
Fortress Elevated Routine Assayed Control Cat No [illegible] (10x5ml)

Fortress Diagnostics Limited, Unit 2C Antrim Technology Park, Antrim, BT41 1QS (United Kingdom)
Tel: +44 (0) 2894 487676 | Fax: +44 (0) 2894 489933 | Website: www.fortressdiagnostics.com

CE [illegible]

Appendix No (7)

fortress diagnostics

HDL CHOLESTEROL

PRODUCT CODE: BXC0422A

CE QUALITY MANAGEMENT SYSTEM ISO 13485 CERTIFIED COMPANY

BXC0422A
2x60ml
STORE AT 2-8°C
INSTRUCTIONS FOR USE
FOR IN-VITRO DIAGNOSTIC USE ONLY

IVD For In Vitro Diagnostics Use Only
LOT Lot Number
REF Catalogue Number
Storage Temperature
Expiry Date (Year / Month)
Warning, Read Enclosed Documents
Instructions For Use
Manufactured By

HDL CHOLESTEROL
PRECIPITANT

Kit Contents:	BXC0422A
R1 HDL Precipitant R4 Cholesterol Standard	2 x 60 ml 1 x 5 ml

Test Principle:
Low density lipoproteins are precipitated by the addition of phosphotungstic acid in the presence of magnesium ions.
The HDL fraction remains in the supernatant and this is determined by cholesterol assay.

Reagent Concentration:

R1 HDL Precipitant	Phosphotungstic Acid	0.55 mmol/l
	Magnesium Chloride	25 mmol/l
R4 Cholesterol Standard		Lot specific (See Vial Label)

Reagent Handling and Preparation:
For Macro assays. The reagents are supplied ready to use and will be stable up until the expiry date when stored at room temperature (20-25°C).
For Semi-micro assays. Pre-dilute the HDL Precipitating reagent (R1) in 4+1 ratio with distilled water i.e. to one of 60ml bottles of HDL precipitating reagent, add 15ml distilled water. This is stable up until the expiry date when stored at room temperature (20-25°C).
Additional Reagents required for Cholesterol CHOD-PAP assay:
BXC0261A (This is not supplied with the BXC0422)
Sample: Serum, Heparinised plasma or EDTA plasma
Manual Procedure:

Wavelength	Temperature	Cuvette	Measurement
500 nm (Hg 546 nm)	20-25°C or 37°C	1 cm light path	Against reagent blank

Precipitation Procedure

	Macro	Semi Micro
Sample	500 µl	200 µl
R1 HDL Precipitant	1000 µl	—
Distilled Water	—	500 µl

Mix and incubate for 10 minutes at room temperature. Centrifuge for 10 minutes at 4000rpm.
Remove the clear supernatant within 2 hours and determine the cholesterol content by the CHOD-PAP method. The supernatant is stable for 2 days at 2-8°C.

Cholesterol CHOD-PAP Procedure:

Only one reagent blank per series. Pipette into test tubes:			
	Reagent Blank	Standard	Sample
DDH2O	100 µl	—	—
Supernatant	—	—	100 µl
Standard R4	—	100 µl	—
Cholesterol Reagent	1 ml	1 ml	1 ml

Mix and incubate for 10 minutes at room temperature or 5 minutes at 37°C. Measure the absorbance of the sample and standard against the reagent blank within 60 minutes.

Calculation:

HDL CHOLESTEROL:
When using a factor:

Wavelength	Macro		Semi-Micro	
	mmol/l	mg/dl	mmol/l	mg/dl
500 nm	4.65	180	5.43	210
Hg 546 nm	7.09	274	8.27	320

When using a Standard:

HDL Conc in the supernatant = $\frac{\text{Abs. sample}}{\text{Abs. standard}}$ x conc of standard

For calibration Fortress HDL cholesterol standard is only used to calibrate the cholesterol assay and should not be diluted in the precipitation step.
The standard value must be multiplied by 3 if using the macro method and by 3.5 if using the semi micro method to compensate for the dilution effect on serum of the precipitation step.

LDL-Cholesterol
LDL-Cholesterol = total cholesterol − $\frac{\text{triglycerides}}{2.2}$ − HDL-Cholesterol in mmol/l
LDL-Cholesterol = total cholesterol − $\frac{\text{triglycerides}}{5.0}$ − HDL-Cholesterol in mg/dl

Linearity:
The method will be linear up to 20 mmol/l (774 mg/dl)

INTERFERENCES:
The assay is unaffected by: Icteric samples (bilirubin < 30 mg/dl), rheumatoid factors <1000 IU/ml, haemolytic samples (Hb < 500 mg/dl) and lipaemic samples (triglyceride < 1200 mg/dl).
Lipaemic samples with a triglyceride concentration >1200 mg/dl should be diluted 1 + 9 with 0.9% (w/v) NaCl before assay. The corresponding result should be multiplied by 10.

As HDL cholesterol is affected by a number of factors such as pregnancy, exercise, hormones, age and sex, each laboratory should establish its own reference ranges.

Expected Values: (NCEP GUIDELINES)

	Low	High
mg/dl	< 40	≥60
mmol/l	<1.04	≥1.55

Use on Automated Analysers:
The HDL cholesterol determination is suitable for use on a range of automated analysers. Specific instructions for these applications are available on request from our technical department.

Quality Control:
It is recommended that a laboratory uses normal and elevated reference sera to verify the performance of any procedure. Results should fall within the specified ranges for the controls.
Fortress Lipid Control Normal & Elevated Cat No BXCO330A/316A
If results fall outside the acceptable range appropriate action as determined by the laboratory's internal quality procedures should be taken.

Health & Safety:
This kit is designed for use by suitably qualified laboratory personnel only. Exercise the normal precautions required for the handling of laboratory reagents. Do not ingest the material. Dispose of material according to local guidelines.

References:
1. Assmann, et al. Clin Chem 1975; 16:451.
2. Tietz NW, Clinical Guide to Laboratory Tests 3rd Edition.
3. NCEP Expert panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults.

Appendix 8

fortress diagnostics

LDL CHOLESTEROL

PRODUCT CODE: BXC0432

CE QUALITY MANAGEMENT SYSTEM ISO 13485 CERTIFIED COMPANY

BXC0432A
2x60ml
STORE AT 2-8°C
INSTRUCTIONS FOR USE
FOR IN-VITRO DIAGNOSTIC USE ONLY

IVD For In Vitro Diagnostics Use Only
LOT Lot Number
REF Catalogue Number
Storage Temperature
Expiry Date (Year / Month)
Warning, Read Enclosed Documents
Instructions For Use
Manufactured By

LDL CHOLESTEROL

Enzymatic (Colorimetric)

Kit Contents:	BXC0432A
R1 LDL Precipitant	2 x 60 ml
R4 Cholesterol Standard	1 x 5 ml

Test Principle:
Low density lipoproteins are precipitated by the addition of heparin at their isoelectric point (pH 5.04). The HDL and VLDL remain in the supernatant and can be determined by enzymatic methods.

LDL Cholesterol = Total Cholesterol – Cholesterol in the supernatant

Reagent Concentration:

R1 LDL Precipitant	Heparin	50,000 IU/l
	Sodium Citrate pH 5.04	0.064 mmol/l
R4 Cholesterol Standard		Lot Specific (See vial label)

Reagent Handling and Preparation:
R1: LDL Precipitant:
The Precipitant reagent (R1) is supplied ready to use and will be stable stored at the recommended temperature until the expiry date quoted.

R4: Cholesterol Standard:
Supplied ready to use and is stable up to the expiry date when stored at 2-8°C.

Reagent required for measurement of Cholesterol:
Cholesterol FAST Cat No. BXC02421A or BXC02618

Sample:
Serum

Manual Procedure:

Wavelength	Temperature	Cuvette	Measurement
546 nm Hg 578 nm	37°C	1 cm light path	Against reagent blank

Precipitation step:
Mix 100 µl of sample with 1000 µl precipitating reagent and keep at room temperature for 10 minutes and then centrifuge at 4000 rpm for 15 minutes. The cholesterol concentration of the supernatant can be determined within 1 hour after centrifugation. Pipette into test tubes as follows:

	Reagent Blank	Standard	Sample
Distilled Water	50 ul	—	—
Standard	—	50 ul	—
Supernatant	—	—	50 ul
Cholesterol Reagent	1 ml	1 ml	1 ml

Mix and incubate for 10 minutes at room temperature or for 5 minutes at 37°C then measure the absorbance of the samples and standard against the reagent blank.
The endpoint will be stable for 1 hour.

Calculation:

Using a Standard:
Concentration of Cholesterol in the supernatant =
Abs. sample / Abs. standard x Conc of standard

Using a factor:
Concentration of Cholesterol in the supernatant =
Sample Absorbance x Factor as follows:

	mmol/l	mg/dl
Hg 546 nm	42.63	1920
500 nm	32.70	1365

The LDL cholesterol result is calculated using the following formula:

LDL Cholesterol = Total Cholesterol − Cholesterol in the supernatant

For Calibration we recommend Fortress Aqueous Cholesterol Standard (Values specified on the vial label). If this standard is used then the results must be multiplied by 11 to allow for the dilution effect of the precipitation step on serum.

N.B. the precipitation step should not be carried out on aqueous standards

Linearity:
The method will be linear up to 20 mmol/l (774 mg/dl)

Expected Values:

	Mg/dl	Mmol/l
Normal	< 100	<2.59
Near or above borderline	100-129	2.59 - 3.35
Borderline high	130 - 159	3.36 -4.12
High	160-189	4.13 - 4.89
Very high	>190	>4.90

Values are supplied as a guideline only.
It is recommended that a laboratory establishes it's own reference ranges, as this can be influenced by many factors. National Cholesterol Education Program (NCEP) Guidelines
LDL cholesterol is affected by a number of factors such as smoking, exercise, hormones, age and sex.

Use on Automated Analysers:
This method is suitable for manual instruments.

Quality Control:
It is recommended that a laboratory uses normal and elevated reference sera to verify the performance of any procedure. Results should fall within the specified ranges for the controls.

Fortress Lipid control Normal and Elevated Cat No BXC0330A & BXC0315A
If results fall outside the acceptable range appropriate action as determined by the laboratory's internal quality procedures should be taken.

Health & Safety:
This kit is designed for use by suitably qualified laboratory personnel only. Assess the normal precautions required for the handling of biological reagents. Do not ingest this material. Dispose of material according to local guidelines.

References:

Appendix no (9):

Frequency of age in the study: