بسم الله الرحمن الرحيم

Sudan University of Science and Technology (SUST)

College of Graduate Studies

The Effect of Blood Cupping on Plasma Creatinine and Uric Acid Levels

تأثير الحجامة الدموية على مستويات البلازما كرياتينين وحمض البوليك

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

By:

Wissam Bushra Mohammed Salih

B.Sc.in Medical Laboratory Sciences - Clinical chemistry

(University of Science and Technology -2014)

Supervisor:

Dr.Abdelgadir Elmugadam

Assistant Professor of Clinical chemistry

August - 2017

Sudan University of Science & Technology

College of Graduate Studies

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

بسم الله الرحمن الرحيم

كلية الدراسات العليا

Ref: SUST/ CGS/A11

Approval Page

(To be completed after the college council approval)

Name of Candidate: Wissam | Bushra | Mohammed | Salih

Thesistitle: the Effect of wet Blood Cupping on Plasma Creatinine and Uric acid Levels

Degree Examined for: M.S.C. in Medical Laboratory Science (Clinical Chemistry)

Approved by:

1. External Examiner

Name: Omaima Ali Mohamed Ahmed

Signature: ..... Date: 20/9/2017

2. Internal Examiner

Name: Nuha Elijaili Abubakr

Signature: ..... Date: 20-9-2017

3. Supervisor

Name: Dr. Abdelgadir Ali Elmugadam

Signature: ..... Date: 20/09/2017

الآيـــــة

قال تعالى:

(ويَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا).

صدق الله العظيم

سورة الإسراء

(الآية رقم 85)

Dedication

To my father who makes me strong …

To my mother who taught me to trust in Allah…

and together pray day and night to make me able to get such success and honor in my life.

To the shining stars in the sky of my life and source of my strength my brothers, sisters and friends.

Acknowledgments

First of all thanks to God that made me powerful to complete this research.I am grateful to my supervisor **Dr**: **Abdelgadir Elmugadam** who encouraged me and I express my gratitude and appreciation to him.

Thanks to **Dr:Mona Jaafer** in the World Center for Prophetic Medicine and Organization of Rawabh which help me in sample collection.

Thank to all the staff of the clinical chemistry department who encouraged and supported me a lot at Sudan University of Science and Technology (SUST) Collage of Graduate Studies. Finally, my best wishes to all people who supported me to prepare this research and give any point of view in making this work.

Abstract

Cupping is an ancient mode of therapy for various ailments, practiced and recommended by ancient healers. This study was conducted to investigate the effect of blood cupping on some biochemical parameters. Fourty peoples were selected randomly between ages 20 to 70 years. Blood samples were collected from World Center for Prophetic Medicine and Organization of Rawabh in Khartoum State. The samples were collected from vein before and ten to fourteen days after cupping of each individual for the analysis of biochemical parameters in the period between February to May 2017. Plasma creatinine and uric acid levels were measured by BS-380 chemistry analyzer and results were analyzed using statistical package for social science (SPSS), computer programmed version 11.5. The study showed that, the mean plasma levels of creatinine was significantly decreased after cupping compared to the level before cupping (1.12±2.17 mmoL/L) (mean±SD:1.42±2.26 mmoL/L) respectively, with p.value (0.000). The mean plasma levels of uric acid before cupping was significantly decreased after cupping (mean±SD:4.33±1.74 mmoL/L) (3.36±1.58 mmoL /L) respectively, with p.value (0.000). Also the finding of this study showed that blood preassure was significantly decreased after cupping (mean±SD: 102.3±13.3 mmHg) (97.6±11.1 mmHg), with p.value (0.008).

Person correlation showed that, there was significant positive correlation between creatinine level and uric acid level in pre and post (r=0.379 , p.value =0.016) (r=0.399 , p.value =0.011) respectively.

The study concluded that , there were significant decreased in the plasma level of creatinine, uric acid and blood preassure in post cupping.

مستخلص الدراسة

الحجامة هي وضع من العلاج القديم لأمراض مختلفة تمارس وأوصت من قبل المعالجين القدماء . أجريت هذه الدراسه للتحقيق في تأثير الحجامة الدموية على بعض المعلومات البيو كيميائيه .

تم اختيار أربعين شخص بشكل عشوائي تتراوح اعمارهم بين سن العشرين الى السبعين عاما و جمعت عينات الدم من المركز العالمي للطب النبوي ومنظمه روابح الخيريه في ولاية الخرطوم .تم جمع العينات من الوريد قبل الحجامة وبعدها بي 10 الى 14 يوما من الحجامة من كل شخص لتحليل المعلومات الكيميائية الحيوية في الفتره ما بين فبراير الى مايو 2017 . 222

تم قياس مستويات البلازما كرياتينين وحمض البوليك باستخدم جهاز التحليل الكيمياى bs-380 والنتائج باستخدام الحزمة الإحصائية للعلوم الاجتماعية (SPSS)، الكمبيوتر المبرمج النسخة 11.5.

أظهرت الدراسة أن متوسط مستويات البلازما من الكرياتينين إنخفضت بشكل ملحوظ بعد الحجامة مانستند : (1.42±2.26) (1.12±2.17) على التوالي وكان الاحتمال الاحصائى للمقارنة 0.000 .

وأظهرت الدراسه أن متوسط مستويات البلازما من حمض البوليك قبل الحجامة إنخفضت بشكل ملحوظ بعد الحجامة ما نستند: (1.74±4.33) (1.58±3.36) على التوالي وكان الاحتمال الاحصائى للمقارنة 0.000 .

كما اظهرت نتائج الدراسه ان ضغط الدم قبل الحجامة إنخفض بشكل ملحوظ بعد الحجامه مانستند :

(13.3±102.3) (11.1±97.6) على التوالي وكان الاحتمال الاحصائى للمقارنة 0.008 .

أظهرت علاقة الارتباط وجود علاقة ارتباط معنويه إيجابيه بين مستوى الكرياتينين و حمض البوليك قبل وبعد الحجامة ما نستند : معامل بيرسون للارتباط قبل الحجامة =(0.379) مستوى المعنوية =(0.016) وبعد الحجامة معامل بيرسون للارتباط =(0.399) و مستوى المعنوية (0.011).

نتسنتج من الدراسة أن هنالك إنخفاض ملحوظ في مستويات البلازما كرياتينين وحمض البوليك و ضغط الدم بعد الحجامه .

List of contents

List of tables:

Lists of figures

List of abbreviations

ADP	Adenosine diphosphate
ATP	Adenosine tri phosphate
BP	Blood preassure
BMI	Body mass index
CHD	Chronic heart disease
CRF	Chronic renal failure
CT	Cupping therapy
CVD	Cardiovascular disease
GFR	Glomuler filtration rate
GIT	Gastro intestinal tract
SAW	Sall allaahu alayhi wasallam
WHO	World health organization

Chapter one

Introduction, Rationale

Objective

1-Introduction, Rationale, Objectives

1.1.Introduction:

Cupping therapy (CT), hijama in arabic, has been practiced in many countries of the world since ancient times. CT is one of the oldest traditional procedures in holistic folk therapy around the world (AL-Shamma and Abdil Razzaq 2009), (Lone et al., 2011). Hijamah comes from the Arabic root word which means “to diminish in volume” and refers to the reduction in blood volume or to the vacuum effect used to draw blood from the body. In the case of the Ahaadeeth (sayings of the Nabi [SAW]) regarding hijamah it refers to the drawing of blood from the body for therapeutic purposes, either to maintain health in the case of one who is not sick or to cure a specific illness or ailment. The vacuum or sucking effect can be achieved by many different methods including sucking with the mouth directly over a cut or wound (as in the case of poisonous bites) using a leech to draw blood, the use of instruments such as animal horns as was done in ancient times or the more modern methods of using bamboo, glass or plastic “cups”, either with fire or a pump mechanism. (Feroz, 2013). There are 2 types of Cupping (hijama) is: dry cupping this is the process of using a vacuum on different areas of the body in order to gather the blood in that area without incisions (small, light scratches using a razor). Wet cupping this is the process of using a vacuum at different points on the body but with incisions in order to remove 'harmful' blood which lies just beneath the surface of the skin (It is recommended that wet cupping is only administered by a cupping therapist) (Chirali, 1999).

Uric acid is a product of the metabolic breakdown of purine nucleotides in the body and its increased circulation in the blood can lead to gout. High levels are also associated with diabetes and the formation of kidney stones. (Feroz, 2013).

Creatinine is derived from the muscle metabolism, where it arises spontaneously and irreversibly by cyclization of creatine and creatine phosphate. Since the amount of creatinine an individual excretes per day is constant (it is directly proportional to muscle mass). Creatinine as an endogenous substance can be used to measure the glomerular filtration rate. (Jan and Klaus , 2005).

1.2. Rationale:

Cupping is one of the oldest and most effective methods of releasing toxins from the body's tissues and organs, is accepted therapeutic practice preferred sometimes for its safety,recently many study paper and researches show the benefits of cupping on blood preassure, uric acid and creatinine which is use as biochemical marker and diagnoses of renal diseases. There is no research conducted or scientific papers published in the Sudan in this subject exactly and very little at the level of world. This research will prove the importance and benefits of blood cupping.

1.3.Objectives:

General objective:

To evaluate the effect of wet blood cupping on plasma creatinine and uric acid levels.

Specific objectives:

1.To measure creatinine, uric acid and blood preassure (pre, post blood cupping).

2.To calculate BMI using hight and weight.

3.To correlate between creatinine and uric acid (pre, post blood cupping).

4.To correlate between BMI, creatinine and age (pre, post blood cupping).

Chapter two

Literature Review

2- Literature review

2.1.Cupping:-

2.1.1.Definition of cupping :

Cupping is one of the oldest and most effective methods of releasing toxins from the body's tissues and organs, in which negative pressure is applied to the skin through sucking cups (dry cupping therapy) (AL- Shamma and Abdil Razzaq, 2009). Cupping is a therapy that stimulates certain points on the body by creating a vacuum in a suction cup, (Rashid and Ashraf, 2015) which helpes in blood removal from the small vessels of the skin and muscles, reducing congestion in the area where cups were applied, (Baghdadi, 2005). The cupping action draws impurities, toxins, pain and inflammation away from the deeper tissues and organs towards the skin where it can be eliminated. The arabic word for cupping (ḥ ijāmah) is derived from the verb ḥ ajama, which means to suck out, and to restore to the previous condition. That is cupping returned the patient to his or her original state of health. (Rashid and Ashraf, 2015). Cupping therapy treats hypertension, neck pain (Wei, 2005), headachechronic hepatitis, ophthalmic diseases, skin diseases and infectious diseases (Kim etal., 2011). In addition ,cupping also treats blood diseases such as hemophilia, hypertension, rheumatic conditions,pain relief ,inflammatory conditions, mental and physical relaxation (Kaleem etal., 2007), migraine headache(Ahmadi etal., 2008) polycythemia ,hemochromatosis (Wright and Finical, 2000), menopause syndrome (Jiang et al., 2004) pain of the knee, liver diseases, renal and uretic colic and other diseases (Akhtar and Siddiqui, 2007). The main purpose of this therapy is to precipitate the circulation of blood and to remove blood stasis and waste from the body (yoo and Tausk , 2004).

2.1.2.Prophetic Sunnah and Prophetic medicine:-

Literally, sunnah means the way, method or style of life. Prophetic sunnah is the well-documented knowledge gained from prophetic hadeeths (sayings), deeds, advices and teachings in all aspects of life related to prophet Muhammad (Mohammad) peace be upon him. Prophetic medicine (in Arabic: Tib Nabawi) is defined as medicine related to Prophet Muhammad peace be upon him. Prophetic medicine dates back to the prophetic era in Makkah and Al-Madinah, two cities in Saudi Arabia (more than 1400 years ago) (Chirali, 1999), (ElSayed, 2013). Anas ibn Maalk reported that the messenger from Allah (SAW) said In: "deed the best of remedies you have is cupping (hijama)…"[Saheeh al-bukhaaree(5371)]. (Bondok, 2010).

Abu Hurayrah (may Allaah be pleased with him) reported that the prophet [SAW] said: "whoever is treated with cupping on the seventeenth, nineteenth or twenty first, will be healed from all diseases." (Reported by Abu Dawood,3861, and al-Bayhaqi, 9/340. The isnad is hasan). In addition to the above days of cupping during the month, cupping for health maintenance is also recommended at the beginning of spring, when the body is emerging from the lethargy of winter, and the flow of body fluids is beginning to increase. Cupping at the beginning of autumn is also recommended as the levels of impurities and toxins in the body have reached their maximum levels. (Bondok , 2010).

2.1.3.History of cupping :-

The history of cupping therapy dated back to thousands of years. (Cao et al., 2010). It is recorded in the books of ahadeeth that amongst other things, such as the use of the turban and miswak, hijamah was a practice of every Nabi. Considering that the Quran clearly states that to every nation a guide was sent, and the fact that at least 124 000 Ambiyaa were sent to this world. Hijamah as a treatment is to be found

throughout the world as a result of this long history of continuous use. The earliest historical evidence of the use of hijamah is from the ancient egyptians. (Feroz, 2013).

Historic evidence shows that cupping was carried out using metal cups, bamboo tree sections or bulls' horn, from which the air was removed by vigorous sucking. Later, this technique gave way to the use of burning tapers or cotton to remove the enclosed air. (Rashid and Ashraf, 2015). In the middle east region it was found that the practice of hijamah was already present before the arrival of the final Rasul (SAW) and the final Nabi (SAW) both encouraged and used it himself on many occasions. Bloodletting managed to survive however into the first part of the 20th century; it was even recommended in a 1923 edition of a textbook called the principles and practice of medicine. During those days, there were four main bloodletting methods practiced by physicians. The first was the continued use of leeches as a bloodletting modality. The second was bleeding of superficial arteries. The third was phlebotomy (also known as "breathing a vein") where a large external vein would be cut in order to draw blood and the last was scarification, a method which involved using tools to make multiple incisions in the skin from which blood was drawn through cupping. (Feroz, 2013).

2.1.4.Main types of cupping :-

Dry Cupping:

A vacuum is created in a glass/plastic cup, which is applied to the skin using a flame or a manual pump. The idea is to draw underlying blood and fluid to the surface of the skin, away from the area of inflammation. This method relieves congestion and also improves blood flow to the site being cupped, thereby facilitating the healing process. (Bondok, 2010).

Wet Cupping:

After the cups are applied, the skin just underneath the cup is cut very lightly several times so that a small amount of blood flows into the cup and can be removed. Wet cupping is used to eliminate excess humours (body fluids) and toxins that cause disease. Between 20-100ml of blood may drain from the area. (Ibn Sina, 2007).

Sliding or Moving Cupping:

This has an effect similar to certain massage techniques. The cups are moved along the surface of the skin while the suction of the skin is active, causing pulling of skin and muscle. This promotes local blood circulation, helps supply more oxygen to the tissues and stimulates the nerves. This is the best method for promoting lymphatic drainage and can be used in the treatment of cellulite and water retention.

All three forms of cupping are well tolerated when practiced by an experienced practitioner. However, after cupping especially wet and dry cupping temporary discolouration of the skin (ecchymosis) occurs which may last for a few weeks. In the case of wet cupping no scarring is left on the area that has been treated. (Bondok, 2010).

2.1.5.Classification 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. The third is method of suction related category which includes fire, manual vacuum, and electrical vacuum cupping therapy. The fourth is based on materials inside cups, and includes herbal, water, laser, moxa, needle, electrical stimulation, and magnetic cupping therapy. The fifth

is area treated related category. The sixth is other cupping methods category that includes sports, cosmetic and aquatic cupping. (Lauche et al., 2011).

2.1.5.1.Category 1:-

Technical type of cupping is in line with technique used in doing cupping.

Dry cupping:

Dry cupping (Lauche et al., 2011) is also given other names such retained cupping (Cao 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 et al., 2011). Negative pressure is the pressure that is less than ambient pressure, and created by exhausting air inside the cup. The practitioners leave the cups on the skin area up to 15 minutes. The pressure inside the cup can be controlled by the number of suctions when using manual pump. Increasing number of suctions will increase the negative pressure inside the cup (Tham et al., 2006). The pressure inside the cup can also be controlled by the fire exposure time when using fire to create negative pressure. Prolonged exposure of the cup to the fire will increase the negative pressure inside the cup that may cause pain or discomfort and may cause skin burn due to the overheating of the cup. Atmospheric (ambient) pressure is higher than the negative pressure inside the cup allowing the skin to pullout. Cupping is applied to increase the circulation of blood and lymph to the local area and also to relieve painful muscle tension. Cupping effectively treats pain and also enhances a patient's general feeling of wellbeing (Lauche et al., 2011). Risk of burn, scar formation and dermatitis are the main disadvantages of this method. (Cao et al., 2012).

Flash cupping :

Flash cupping (Cao et al., 2012), also referred to as empty cupping (Cao et al., 2010) is the name given when several medium to light pressure cupping are preformed several times in quick succession along the area being considered for

treatment that requires stimulation (Cao et al., 2010). It only takes less than 30 seconds from the time when cup is applied and then removed because it entails stimulation process. It is done by using one cup or some practitioners use four medium sized cups. They apply the four cups quickly then reapply them on the skin of next area before 30 seconds and used to stimulate para spinal lines on the back. This method is used when dry cupping is not indicated especially in young people and ladies. (Kim et al., 2011).

Wet cupping:

Wet cupping (Kim et al., 2011), has been given several other names: full cupping (Al-Rubaye, 2012), bloodletting cupping (Ahmed etal., 2004) and bleeding cupping. (Chen and Zheng, 2010). This method is used most frequently in traditional medicine (Cao et al., 2010). Asurgical instrument is used to scrape the skin and the cup is then applied to suck blood (Ahmed et al., 2004). It may help in treatment of chronic musculoskeletal pain (Kim et al., 2011), skin disinfection. The risk of infection, vasovagal attacks and scars are the main disadvantages of this method. (Jiang et al., 2004).

Massage cupping:

Also known as moving cupping (Cao et al., 2010), dynamic cupping (Winkes et al., 2012) and gliding cupping. Is a method of massage and done by applying oil to the skin and moving the cup by a weak suction on the area that needs massage (Tham et al., 2006). Various types of oils may be used such as olive oil, peppermint oil and lavender oil. It is suitable for all people even young and elderly people. The cost may be the disadvantages of this method. (AL-Shamma and Abdil Razzaq, 2009).

2.1.5.2.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. (AL-Shamma and Abdil Razzaq, 2009).

Light cupping:

Practitioners produce a weak suction in the cup to do light cupping. (AL-Shamma and Abdil Razzaq, 2009). The pressure inside the cup is between 100 and less than 300 millibar which is a unit of atmospheric pressure. It is suitable for children and elderly people (Tham et al., 2006) and in sensitive body parts like the face. Light cupping pressure used in massage, dry and flash cupping techniques and used to treat pain disorders for elderly people and facial massage. 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. (AL-Shamma and Abdil Razzaq, 2009).

Medium cupping:

Medium cupping (AL-Shamma and Abdil Razzaq, 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 et al., 2006) used to treat musculoskeletal pain conditions, headaches and to increase blood circulation. Frequently observed cupping marks is one of its disadvantage. (AL-Shamma and Abdil Razzaq, 2009) .

Strong cupping:

Strong cupping is done by creating high negative pressure inside the cups. (AL-Shamma and Abdil Razzaq, 2009). 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 et al.,

2006). The risks of dermatitis and skin burn are the two main disadvantages of this method. (Teut et al., 2012).

Pulsatile cupping thera:

Pulsatile cupping is special type of cupping therapy. The pressure inside the cups is not constant but variable, varies between 100 and 200 millibar, at the interval of 2 seconds. It is used in randomized clinical trials evaluating the efficacy of cupping therapy in the treatment of osteoarthritis. (Teut et al., 2012).

2.1.5.3.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. (Huang et al., 2011).

Fire cupping therapy:

Fire cupping is a type of cupping done by creating negative pressure inside the cups by using fire. 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. The cup is applied immediately on the skin surface. (Huang et al., 2011). There is a risk of skin burn in this cupping type because of using fire. (Duh and Chiu, 2015).

Manual vacuum cupping therapy:

Has also other names: vacuum cupping and opening cupping. (Huang et al., 2011). It is done by creating negative pressure inside the cups by using manual suction pump. (Duh and Chiu 2015). The main advantages of this method are: 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 et al., 2011) .

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. 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 and Chiu, 2015)

2.1.5.4.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. (Duh et al., 2015).

Needle cupping:

Needle cupping is done by applying the acupuncture needle first and then the cup is applied over it. (Duh 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. (Anees et al., 2015).

Hot cupping:

Dried herb, called Moxa is used to do hot cupping (Anees et al., 2015) or Moxa cupping (Cao 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. Observing patient during the procedure is very important because the patient is at risk of burn, which is the main disadvantage of this method. (Wu et al., 2013).

Herbal cupping:

Herbal cupping (Wu et al., 2013) or medicinal cupping (Cao et al., 2011) is done by boiling cups in a suitable herbal tincture and then applied to the skin (Wu 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. Cups were left for 10 minutes. One session daily and the entire treatment requires 15 sessions. Caution is taken to minimize the risk of burns or blisters from the hot cups or hot water dripping onto the skin (Cao et al., 2011).

Magnetic cupping therapy:

Is done by using magnetic cupping sets that contains magnets inside the cups. Electromagnetic stimulation increases the therapeutic effectiveness of cupping, especially when applied to joints including the knees and elbows. It is used in the treatment of diseases related to joints (Chirali and Ilkay, 2014).

Laser cupping therapy:

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

Electric 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 (Chirali and Ilkay 2014). The advantage of this method is the dual effect of electrical stimulation and cupping therapy (Al-Rubaye, 2012).

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 (Al-Rubaye, 2012). Water cupping is especially beneficial for treating asthma and related conditions including dry cough (Chirali and Ilkay, 2014).

2.1.5.5.Category 5:

Area treated related types :

This category of cupping types is classified according to the body part considered for the cups application (Shaban, 2013).

Pedi cupping :

Pedi Cupping is a combination of reflexology, massage cupping and plantar fascial release on the leg and foot (Shaban, 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 (Chirali and Ilkay 2014).

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, circling the umbilicus clockwise and then increasing the circle. It begins with flash cupping and continues with massage cupping on abdomen (Chirali and Ilkay, 2014). It stretches the walls of the organs, increase blood circulation and promote the digestive system (Shaban, 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, 2003).

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 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 2014).

Female cupping:

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

Male cupping :

Male cupping is the use of vacuum erection device to stimulate and support erection function. (Shaban, 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. (Chirali and Ilkay, 2014), (LaCross, 2014).

2.1.5.6.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.

Sports cupping :

Cupping is used for the treatment of sports and athletic injuries and for rehabilitation purposes. (Chirali and Ilkay, 2014) (LaCross, 2014). One of the best examples of it is the treatment of hamstrings conditions by cupping (LaCross, 2014).

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. Small size cups are used for facial cupping and large cups are used for the arms and legs. (Chirali and Ilkay, 2014)

Aquatic cupping:

Aquatic cupping is doing cupping underwater. Muscles tend to stretch much underwater and doing cupping may help in this situation. Aquatic cupping is water-based treatments of therapeutic value. It is used for rehabilitation and musculoskeletal diseases. (Baguanfa treatments, 2005).

2.1.6. Benefits of cupping:

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. 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. Localized 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. Cupping exerts a beneficial effect up to ten centimetres into the tissue it is applied to, so compelling them to release the toxins they hold. In addition, it stimulates local lymphatic circulation, so enhancing their primary effect, namely, the mobilization and removal of toxic waste material. (Rashid and Ashraf, 2015).

2.1.7.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 (injury, headaches ,sihr (black magic) , poison). These four conditions mentioned in the Ahadeeth for which the Nabi (SAW) had Hijamah done give us an indication as to what type of diseases hijamah is useful for :

1.External injuries.

2.Internal disorders which are either due to heat, poor circulation or build up of toxins.

3.Sihr (black magic).

4.Poison (this can also be natural poisons such as heavy metal toxicity etc.).

(Feroz, 2013).

2.1.8.Hijamah in the condition of strength:

The simplest method of Hijamah is that used for general health promotion and the Nabi (SAW) used the most common method. The areas used in this general form of hijamah are. For a man: the area between the shoulder blades, most commonly in line with the inferior end of the scapula which is in line with the 7th thoracic vertebra, the occipital area of the neck in the recesses formed between the upper portion of the sternocleidomastoid and the trapezius muscles, on the head in the midline and on the anterior aspect of the foot in a depression distal to the junction of the 2nd and 3rd metatarsal bones. In women the same areas are used except for the hijamah point on the head but the quantity of blood removed is less. (Feroz, 2013).

2.2.Creatinine:-

Creatinine is a waste product formed in muscle from a high-energy storage compound, creatine phosphate. Creatine phosphate can be stored in muscle at

approximately four times the concentration of adenosine triphosphate. In muscles it spontaneously undergoes degradation to form a cyclic anhyride-creatinine. The blood concentration of creatinine and its excretion in urine are remarkably constant in healthy individuals. Therefore serum creatinine level is used as an indicator for assessing kidney function. (Ray and Suzanne, 2012) .

Creatinine is freely filtered at the glomerulus. Its concentration is inversely related to GFR. As a GFR marker it is convenient and cheap to measure but its concentration in the plasma is affected by age, gender, exercise, muscle mass, certain drugs, for example cimetidine, trimethoprim, and nutritional status. Creatine in meat is converted to creatinine when it is cooked. Once eaten, it increases the creatinine concentration in plasma after ingestion. (Nessar, 2011)

Serum creatinine concentration is related to muscle mass and the values are lower in children. Increased serum creatinine is associated with decrease in glomerular filtration rate (GFR), whether the cause is prerenal, renal or postrenal. Prerenal factors include conditions such as congestive heart failure, shock, diarrhoea, uncontrolled diabetes mellitus, use of diuretics and so on. Renal factors involve mainly damage to the glomeruli. Postrenal factors may be prostatic hypertrophy, calculi blocking the ureters or neoplasms compressing the ureters. The serum creatinine concentration is monitored closely after a renal transplantation because a rising concentration,even though small, may be an indication of g graft rejection. **(**Ray and Suzanne, 2012).

2.2.1.Creatinine Metabolism :-

Creatine is synthesized in the liver, pancreas and kidneys from the amino acids arginine, glycine and methionine. Creatine is transported through the circulatory system to muscle, brain and other organs, where it is converted to phosphocreatine and acts as an energy reservoir much like ATP. Creatinine is produced as awaste product of creatine and phosphocreatine. Because much of the creatinine is

produced in muscle, the amount of creatinine that is measured in blood is proportional to the patient's lean muscle mass. The waste product creatinine, enters the blood supply where it is removed through the kidneys. (Davis, 2007) .

Creatine (N-methylguanidoacetic acid) and its phosphorylated form creatine phosphate (a guanidophosphate) serve as an ATP buffer in muscle metabolism. In creatine phosphate the phosphate residue is at a similarly high chemical potential as in ATP and is therefore easily transferred to ADP. Conversely, when there is an excess of ATP, creatine phosphate can arise from ATP and creatine. Both processes are catalyzed by creatine kinase. In resting muscle, creatine phosphate forms due to the high level of ATP. If there is a risk of a severe drop in the ATP level during contraction, the level can be maintained for a short time by synthesis of ATP from creatine phosphate and ADP. In a nonenzymatic reaction small amounts of creatine and creatine phosphate cyclize constantly to form creatinine, which can no longer be phosphorylated and is therefore excreted with the urine . Creatine does not derive from the muscles themselves, but is synthesized in two steps in the kidneys and liver (left part of the illustration). Initially, the guanidino group of arginine is transferred to glycine in the kidneys yielding guanidino acetate. In the liver N-methylation of guanidino acetate leads to the formation of creatine from this. The coenzyme in this reaction is S-adenosylmethionine. (Jan and Klaus, 2005).

2.2.2.Creatinine secretion :-

Creatinine is actively secreted by the renal tubules and, as a result, the creatinine clearance is higher than the true GFR. The difference is of little significance when the GFR is normal, but when the GFR is low (<10 mL/min), tubular secretion makes a major contribution to creatinine excretion and creatinine clearance significantly overestimates the GFR. The effect of creatinine breakdown in the gut also becomes significant when the GFR is very low. Certain drugs, including

spironolactone, cimetidine, fenofibrate, trimethoprim and amiloride, decrease creatinine secretion and thus can reduce creatinine clearance. (William, 2012).

2.2.3.Plasma creatinine:-

Plasma creatinine concentration is the most reliable simple biochemical test of glomerular function. Ingestion of a meat-rich meal can increase plasma creatinine concentration by as much as 20 μmol/L for up to 10 h afterwards, so ideally blood samples should be collected after an overnight fast. Strenuous exercise also causes a transient, slight increase in plasma creatinine concentrations. Plasma creatinine concentration is related to muscle bulk and therefore a value of 120 μmol/L could be normal for an athletic young man but would suggest renal impairment in a thin, 70-year-old woman. Although muscle bulk tends to decline with age, so too does the GFR, and hence plasma creatinine concentrations remain fairly constant. Some commonly used laboratory methods for the measurement of creatinine can suffer from interference, for example from bilirubin and ketones. The laboratory should be able to advise on whether this may be a problem in individual cases. The reference range for plasma creatinine in the adult population is 60–120 μmol/L, but the day-today variation in an individual is much less than this range. GFR can decrease by 50% before plasma creatinine concentration rises beyond the normal range. Changes in plasma creatinine concentration can occur independently of renal function owing to changes in muscle mass. Thus a decrease can occur as a result of starvation and in wasting diseases, immediately after surgery and in patients treated with corticosteroids, an increase can occur during refeeding. However, changes in creatinine concentration for these reasons rarely lead to diagnostic confusion.In pregnancy, the GFR increases. This usually more than balances the effect of increased creatinine synthesis during pregnancy and results in a decrease in plasma creatinine concentration. (William, 2012).

2.2.4.Glomerular filtration rate (GFR) and Clearance :-

This is the volume of blood filtered per unit time by all glomeruli combined, approximately 125mL per minute (or 7.5 L/h). **(**Ray and Suzanne 2012). Creatinine clearance measures the ability of the glomerulus to filter chemicals from the blood. The best substance to use for glomerular clearance would be a chemical that is filtered completely through the glomerulus and not reabsorbed through the nephron tubule. Creatinine meets that criterion for clearance and is also an endogenous substance that is it is produced in the body. Other substances such as inulin, may be used for evaluation of creatinine clearance however, they are exogenous substances and must be introduced into the body. Creatinine clearance is measured as a rate therefore, the test must be timed. The test measures the movement of the substance from blood to urine therefore, both blood and urine concentrations of the chemical must be measured. Error may be encountered such factors as increased tubular reabsorption of creatinine reduced creatinine generation from muscle tissue and dietary changes in nitrogenous compounds may affect creatinine clearance. (Davis, 2007).

2.3.Uric acid:

Uric acid is the excretory end product of purine metabolism in humans. It is removed from the blood by filtration through the kidneys and excreted in urine. The excretion and poor solubility of uric acid ensure that it is normally present in only small concentrations in the plasma. A number of disorders associated with purine metabolism are predominantly the result of an abnormal catabolism. This either increases the amount of uric acid formed or decreases its excretion, which results in hyperuricaemia. The clinical outcome ranges from relatively mild, for example gout, to severe symptoms such as mental retardation and even death. (Nessar, 2011).

2.3.1.Purine catabolism and excretion:

Purine nucleotides are catabolized (degraded) by reactions that initially form their respective nucleosides: inosine, adenosine, guanosine and xanthosine. The commonly found nucleotides are eventually converted by a variety of enzyme catalysed reactions into xanthine. In turn, xanthine is oxidized to uric acid. Consequently, uric acid is the main end product of metabolism and the degradation of purines. Uric acid is sparingly soluble but despite this the kidneys play the major role in removing it from the blood. This is possible because it can ionize in the presence of sodium giving the salt monosodium urate. Monosodium urate is generally referred to as uric acid in clinical environments and we will adhere to this convention. Approximately 98% of the uric acid filtered by the glomeruli is reabsorbed by the proximal tubules. Despite this, about two-thirds to three-quarters of the uric acid excreted is eliminated by the kidneys. The remaining uric acid is secreted into the gastrointestinal tract (GIT) where it is metabolized by gut bacteria in a process called uricolysis to form carbon dioxide and ammonia.(Nessar, 2011).

2.3.2.Uric acid metabolism :-

Purine nucleotides are essential components of nucleic acids: they are intimately involved in energy transformation and phosphorylation reactions and act as intracellular messengers. There are three sources of purines in humans: the diet, degradation of endogenous nucleotides and de novo synthesis. As purines are metabolized to uric acid, the body urate pool (and hence plasma concentration) depends on the relative rates of both urate formation from these sources and urate excretion. Urate is excreted by both the kidneys and the gut, renal excretion accounting for approximately two-thirds of the total. Normal urate clearance is about 10% of the filtered load. In normal subjects, urate excretion increases if the filtered load is increased. In chronic kidney disease, the plasma concentration rises

only when the glomerular filtration rate falls below about 20 mL/min. (William, 2012).

2.3.3.Disorder associated with uric acid:-

The concentrations of uric acid in blood are normally determined in samples of serum.Concentrations are generally higher in males than females with a usual reference range for males of 0.1–0.42 and for females 0.1–0.36 mmol/L. Values below or above these limits are called hypouricaemia and hyperuricaemia respectively. (William, 2012).

2.3.3.1.Hypouricaemia:

Hypouricaemia is a measured serum concentration of uric acid below its reference ranges. It is rare, being associated with relatively few clinical conditions. The primary metabolic cause is an autosomal recessive disorder in which there is a deficiency of xanthine oxidase activity in the liver. This results in increased excretions of xanthine and hypoxanthine and the formation of xanthine stones. This condition is called xanthinuria. Hypouricaemia can be caused by treatment with the drug allopurinol that inhibits xanthine oxidase thus reducing the synthesis of uric acid.(Nessar, 2011). Also, severe liver disease and renal tubular disorders such as the Fanconi syndrome. and the use of uricosuric drugs such asprobenecid. **(**William, 2012).

2.3.3.2.Hyperuricaemia:

Hyperuricaemia is defined as an increase in the concentration of uric acid in samples of serum from patients to values above the reference ranges. The concentration of uric acid in plasma reflects an equilibrium between the amount ingested and produced and the quantity excreted, then hyperuricaemia may be due to an increase in its formation or areduction in its excretion or a combination of both. The causes of hyperuricaemia are divided into primary disorders due to inherited metabolic diseases involving purine metabolism or secondary ones

caused by a co-existing clinical condition. Both of course, lead to an accumulation of uric acid in the body. (Nessar, 2011).

Primary causes of hyperuricaemia:

More than 99% of causes of primary gout are idiopathic, that is there is no known cause.Although contributory factors such as hormones, family history and dietary causes are implicated. The X-linked recessive disorder, Lesch-Nyhan syndrome is characterized by an increase in the de novo synthesis of purines and is a major cause of primary hyperuricaemia. Lesch-Nyhan syndrome is a rare condition and affects only 1 in 10,000 to 1 in 380,000 live births. It is characterized in infants by poor feeding, severe developmental delay, and self mutilation.

Von Gierke's disease or glycogen storage disease type 1 is caused by a deficiency in glucose-6-phosphatase (G6P) activity, which indirectly affects the de novo synthesis of purines. This recessive condition presents in infancy with symptoms of hypoglycaemia, failure to thrive and hyperuricaemia. (Nessar, 2011).

Secondary causes of hyperuricaemia :

An increase in the concentration of uric acid in plasma may be secondary to an increase in the uric acid formed in the body. Thus, secondary hyperuricaemia may result from a number of factors such as a high dietary intake, increased metabolism of ATP, or an increase in the turnover of nucleic acids,cytotoxic drugs, alcohol, myeloproliferative disease, Polycythaemia. (Nessar, 2011).

Hyperuricemia can be treated with allopurinol, a competitive inhibitor of xanthine oxidase. (Jan and Klaus, 2005). The fact that purine degradation in humans already stops at the uric acid stage can lead to problems, since in contrast to allantoin,uric acid is poorly soluble in water. When large amounts of uric acid are formed or uric acid processing is disturbed, excessive concentrations of uric acid can develop in the blood (hyperuricemia). This can result in the accumulation of

uric acid crystals in the body. Deposition of these crystals in the joints can cause very painful attacks of gout. (Jan and Klaus, 2005).

2.3.3.3.Gout:

Gout is the commonest crystal deposition disease and occurs when abnormal uric acid metabolism leads to deposition of sodium urate crystals in joints, soft tissues and the urinary tract. It is 10 times more common in men than in women. The underlying problem is hyperuricaemia or increased levels of uric acid, due to its overproduction or decreased renal excretion. The latter is more commonly responsible and may be seen in chronic renal disease with high blood pressure, with alcoholism or with the use of thiazide diuretic drugs. Overproduction of uric acid may occur as a result of increased purine production, since urate is a product of purine metabolism; purines are increased in myeloproliferative diseases such as leukaemia or polycythaemia rubra vera, psoriasis or carcinoma. Acute gout causes a sudden onset of severe pain, redness and swelling in a joint, usually the joint in the big toe. Itmay affect multiple joints, and crystals may also be deposited in cartilage, most notably around the ear. Examination of aspirated synovial fluid from infected joints shows the long, pointed urate crystals and treatment in the short term is with nonsteroidal antiinflammatory drugs, injection of corticosteroids into affected joints. In the longer term, patients who are overweight should be advised to lose weight, alcohol consumption should be decreased; drugs to inhibit purine breakdown (e.g. allopurinol) or drugs to promote the renal excretion of uric acid (e.g. probenecid) can also be used. (Ray and Suzanne, 2012)

Gout can be precipitated by a sudden change (either increase or decrease) in urate concentration. When urate concentration has fallen rapidly in a hyperuricaemic individual (e.g. as a result of achange in diet, decrease in alcohol consumption or treatment with a hypouricaemic drug) the plasma urate concentration may not be

elevated when the patient presents with gout. The solubility of monosodium urate declines rapidly with decreasing temperature and this may to some extent explain the tendency for the more peripheral joints, which have lower intra-articular temperatures to be more frequently affected. Monosodium urate crystals forming in joints are engulfed by neutrophil leukocytes but damage the lysosomal membranes of these cells, so causing cellular disruption. The generation of superoxide free radicals and release of lysosomal enzymes into the joint precipitates an acute inflammatory reaction. The release of interleukins (particularly IL-1β) and other inflammatory mediators from monocytes and tissue macrophages also provides an inflammatory stimulus. (William, 2012).

Gout is customarily defined as primary (idiopathic) or secondary (when a condition known to cause hyperuricaemia is present). However, gout is uncommon when hyperuricaemia develops secondarily to other conditions. The tendency for hyperuricaemia and gout to be familial has led to investigation for a causal inherited metabolic defect. Although there are a few rare conditions in which such adefect does lead to hyperuricaemia, none has been found in the great majority of cases of primary gout. Some 90% of patients appear to excrete urate at a rate inappropriately low for the plasma concentration, while about 10% have excessive urate production. Clearly defined inherited disorders are responsible for fewer than 1% of cases. Dietary factors and alcohol ingestion exacerbate hyperuricaemia in about 50% of patients, but while their amelioration may reduce the plasma urate concentrations somewhat, these usually remain elevated. Gout is rare in women before the menopause, but the incidence increases markedly thereafter. Patients with gout frequently have hyperlipidaemia (particularly hypertriglyceridaemia) and often other features of the metabolic syndrome. Hyperuricaemia is associated with the resistance to insulin that is characteristic of this syndrome. However, although

the syndrome itself is an important risk factor for cardiovascular disease, it is uncertain whether this is the case with hyperuricaemia alone. (William, 2012).

Diagnosis of gout: a diagnosis of gout usually begins with a review of the family medical history and a physical examination. (Nessar, 2011). But is supported by the demonstration of hyperuricaemia and can be confirmed by the presence of tophi or of monosodium urate crystals in the synovial fluid. (William, 2012). A swollen joint and red shiny skin above the affected area can indicate gout. However, a thorough medical examination is required to exclude other conditions such as rheumatoid arthritis, or infection pseudogout psoriatic arthritis. Most patients with gout have concentrations of uric acid in samples of their serum above their reference ranges. (Nessar, 2011).

Pseudogout: calcium pyrophosphate crystals can also be deposited in tissues, mainly in articular cartilage of the knee, causing pseudogout. The cause is unknown, but ittends to occur in older people with equal incidence in men and women. It may follow primary hypoparathyroidism, gout, haemochromatosis or hypothyroidism. In pseudogout, microscopy of aspirated synovial fluid shows small, rectangular crystals of calcium pyrophosphate (which show birefringence under polarized light. Treatment is with joint aspiration, non steroidal anti inflammatory drugs and corticosteroids. **(**Ray and Suzanne, 2012) .

2.4.Body mass index (BMI) :-

Obesity can be evaluated as the body mass index (BMI). This is calculated as weight in kilograms divided by height in metres squared (kg/m2). A normal BMI is 20–25 kg/m2. People with a BMI in the range of 25–30 kg/m2 are described as being overweight, whilst a BMI higher than 30 kg/m2 is regarded as obese. A BMI more than 40 kg/m2 is regarded as morbidly obese. Diabetes risk increases with rising BMI. The risk curve starts to rise at a BMI of 22.5 kg/m2, which is normal, and from 25–30 kg/m2 the risk doubles. (Nessar, 2011)

2.5.Blood preassure :-

Blood pressure, like height and weight, is a continuous biological variable with no cut-off point separating normotension from hypertension. Therefore, a definition of hypertension is usually taken as that level of arterial blood pressure associated with doubling of long-term cardiovascular risk. (Kaplan, 2002)

Normal blood pressure is defined as levels <120/80 mmHg. Systolic blood pressure of 120–139 mmHg or diastolic blood pressure 80–89 mmHg is classified as prehypertension. Hypertension is defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg.

Hypertension is divided into two stages: stage 1 includes patients with systolic blood pressure 140–159 mmHg or diastolic blood pressure 90–99 mmHg, stage 2 includes patients with systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥100 mmHg. (WHO, 2005).

Chapter Three

Materials and Methods

3.Materials and Methods

3.1.Study approach

A quantitative method was used to evaluate the plasma creatinine and uric acid in pre and post wet blood cupping during the period from February to May 2017.

3.2.Study design and Study area

This experimental study was conducted in Khartoum state, the capital of Sudan.

3.3. Study population and Sample size

The study included fourty volunteeres aged between 20-70 years who were selected randomly to participate in this study.

3.4.Inclusion criteria

Study included any individual that come to blood cupping .

3.5.Exclusion criteria

Any individual don't do cupping was excluded and any patients have missing data in pre or post cupping were excluded.

3.6.Ethical considration

Consent was taken regarding acceptance to participate in the study and re-assurance of confidentialty. Before the specimen was collected, the donors knew that this specimen was collected for research purpose.

3.7.Data collection

Data were collected using a structural interviewing questionnaire, which was designed to collect and maintain all valuable information concering each case examined.

3.8.Sample collection and processing

About 5 ml of venous blood were collected from each participant in pre and post cupping. The samples were collected under aseptic conditions and divided in two sterile lithium heparin containers and centrifuged for 5 minutes at 3000 RPM to obtain

plasma then the obtained samples were kept in plain containers at -70 C^0 until the time of analysis.

3.9.Biochemical testing:

The laboratory analysis of samples was performed at Omdurman teaching hospital central laboratory by using BS-380 chemical analyzer (BS-380 is a full automated chemical analyzer produced by mindray which provide 300 test per hour).

The methods that were used in this study are the chemical (Jaffe's) for creatinine measurement and enzymetic (uricase/peroxidase) method for uric acid measurement.

3.9.1.Creatinine measurement :-

3.9.1.1.Principle of method:

Creatinine in the sample react with picrate in alkaline medium forming a coloured complex. The complex formation rate is measured in ashort period to avoid interferences (bartels , fabiny 1971). Seurm and plasma sample contain proteins that react in anon specific way never theless, the result can be corrected subtracting afixed value.The use of this correction is known as the jaffe method compensated. (weber, 1991) (peake , 2006)

3.9.1.2.Procedure of creatinine measurement (Appendix II)

1.The working reagent and photometer was brought to 37ºC,Pipette into a cuvette:

	Test tube	**Standard tube**	**Blank tube**
Reagent	**1000**ul	**1000**ul	**1000**ul
Serum /plasma	10 microliter		
Standard		10 microliter	

2. The cuvette was mixed and inserted into the photometer, Start stopwatch.

3. The absorbance was recorded at 500 nm after 30 seconds (A1) and after 90 seconds (A2) of the sample and standard then creatinine concentration in the sample was calculated using the following general formula:

[(A2 – A1) Sample /(A2 – A1) Standard] x C Standard x Sample dilution factor – Corrective Factor = C Sample (weber , 1991) (peake , 2006)

3.9.2.Uric acid measurement:

3.9.2.1.Principle of the method:

Uric acid in the sample originates, by means of the coupled reactions described below:

A coloured complex that can be measured by spectrophotometry (Barham **,** Trinder 1972) (Fossati , Prencipe , Berti .1980).

Uric acid + O2 + 2 H2O *uricase* Alantoin + CO2 + H2O2

2 H2O2 + 4 – Aminoantipyrine + DCFS *peroxidase* Quinoneimine + 4 H2O

3.9.2.2.Procedure of uric acid measurement (Appendix III)

1.The reagent was brought to room temperature.

2. Pipette into labelled test tubes:

	Blank	**Standard**	**Sample**
Distilled water	25 ul		
Uric Acid Standard (		25 ul	
Sample			25 ul
Reagent	**1000 ul**	**1000 ul**	**1000 ul**

3.The tubes was mixed thoroughly and incubated for 10 minutes at room temperature (16-25ºC) or for 5 minutes at 37ºC.

4.The absorbance (A) of the Standard and the Sample was measured at 520 nm against the Blank.The colour was stable for at least 30 minutes.

Then uric acid concentration in the sample was calculated using the following general formula:

[A Sample /A Standard] x C Standard x Sample dilution factor = C Sample

3.10.Quality control:

The precision and accuracy of all methods used in this study were checked by commercially prepared control sample before it is application for the measurement of test and control samples.

3.11.Statistical analysis :

Data was analyzed to obtain mean, standard deviation and correlation of the sampling using statistical packag for social science (SPSS) computer programed version 11.5

T test and person correlation were used for comparison and correlation .

Chapter Four

Results

4.Results

Table (4-1): The clinical and biochemical characteristics of this study subjects were a comparison between the baseline (befor cupping) and 10-14 days after cupping. Blood pressure was significantly decrease after cupping compared to 10-14 days after cupping of (p.value = 0.008) as well as plasma level of uric acid and creatinine also they was significant decreased (p.value = 0.000 and p.value = 0.000) respectively.

Table (4-2): Illustrate the correlation (P.value , r) of age with creatinine in pre and post cupping(P.value=0.582, r=-0.090),(P.value=0.432, r=-0.128) respectively.

Correlation of age with uric acid in pre and post cupping (P.value =0.929,r=0.015),(P.value=0.458,r=-0.121) respectively.

Correlation of age and blood pressure in pre and post cupping (P.value=0.997,r=-0.001),(P.value=0.962,r=-0.009) respectively.

Correlation between creatinine and uric acid in pre (r=0.379 ,p.value =0.016) and post (r=0.399, p.value =0.011) .

Correlation between BMI and uric acid in pre (r=0.133, *P.value* =0.415) and post(r=0.151, *P.value* =0.353)

Figure (4-1): Show correlation between creatinine concentration and uric acid concentration in pre cupping (r= 0.379, *P.value* = 0.016).

Figure (4-2): Show correlation between creatinine concentration and uric acid concentration in post cupping (r= 0.399, *P.value* = 0.011).

Figure (4-3): Show correlation between age and BMI (r= 0.442, *P.value* = 0.004).

Figure (4-4): Show correlation between creatinine concentration in pre cupping and post BMI (r= -0.043, *P.value* = 0.790).

Figure (4-5): Show correlation between creatinine concentration in post cupping and post BMI (r= -0.051, *P.value* = 0.756).

Figure (4-6): Show correlation between blood pressure in pre cupping and post BMI (r= 0.347, *P.value* = 0.060).

Figure (4-7): Show correlation between blood pressure in post cupping and post BMI (r= 0.262, *P.value* = 0.161).

Table (4-1): Illustrate the mean concentrations of creatinine, uric acid and blood pressure in pre and post cupping person .

Variable	Pre N=40 Mean±SD	Post N=40 Mean±SD	*P.Value*
Creatinine mmol/l	1.425±2.26	1.128±2.17	0.000
Uric acid mmol\l	4.333±1.74	3.367±1.587	0.000
Blood pressure mm.Hg	102.350±13.30	97.65±11.199	0.008

*Result given in mean ± SD, *P-value* ≤ 0.05 Considerd significant.

* Paired sample T test was used for comparison.

Table (4-2): Illustrate the person correlation (p.value, r) of creatinine,uric acid in pre and post cupping . Age with creatinine, uric acid and blood preassure in pre and post cupping.

Variable	Person correlation (r)	p.value
Creatinine with uric acid in pre cupping	0.379	0.016
Creatinine with uric acid in post cupping	0.399	0.011
Age with creatinine in pre cupping	-0.090	0.582
Age with creatinine in post cupping	-0.128	0.432
Age with uric acid in pre cupping	0.015	0.929
Age with uric acid in post cupping	-0.0121	0.458
Age with blood pressure in pre cupping	-0.001	0.997
Age with blood pressure in post cupping	-0.009	0.962
BMI with uric acid in pre cupping	0.133	0.415
BMI with uric acid in post cupping	0.151	0.353

*Result given as p.value and r small

**P-value* ≤ 0.05 Considerd significant.

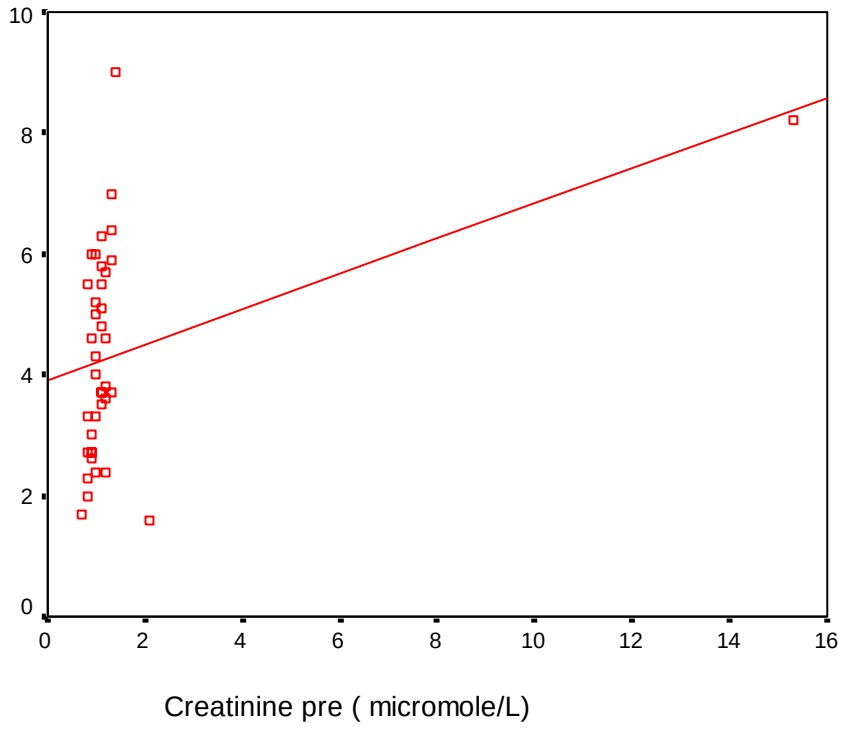


Figure (4-1): Show correlation between creatinine concentration and uric acid concentration in pre cupping (r= 0.379, *P.value* = 0.016).

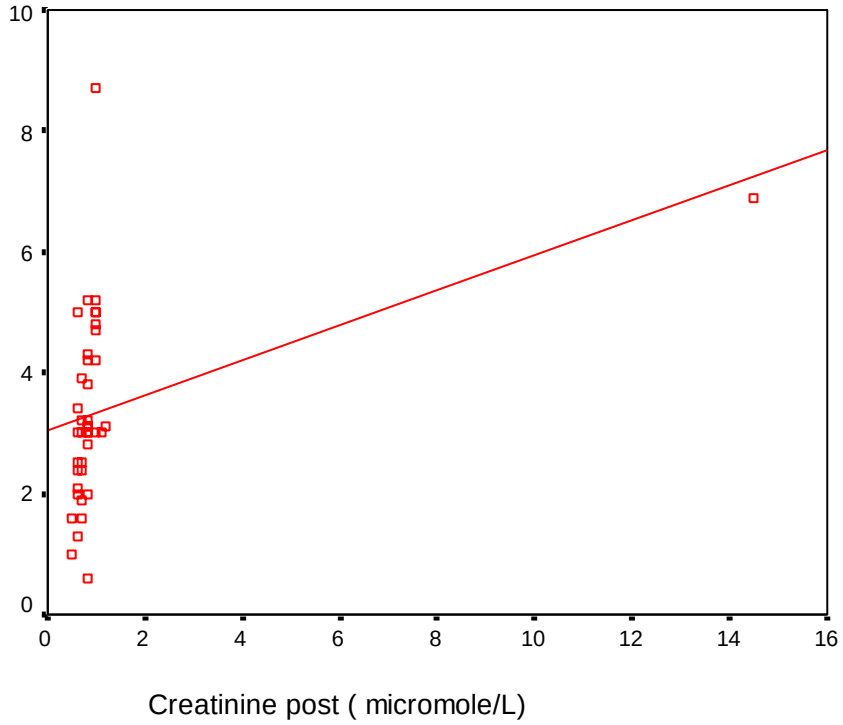


Figure (4-2): Show correlation between creatinine concentration and uric acid concentration in post cupping (r= 0.399, *P.value* = 0.011).

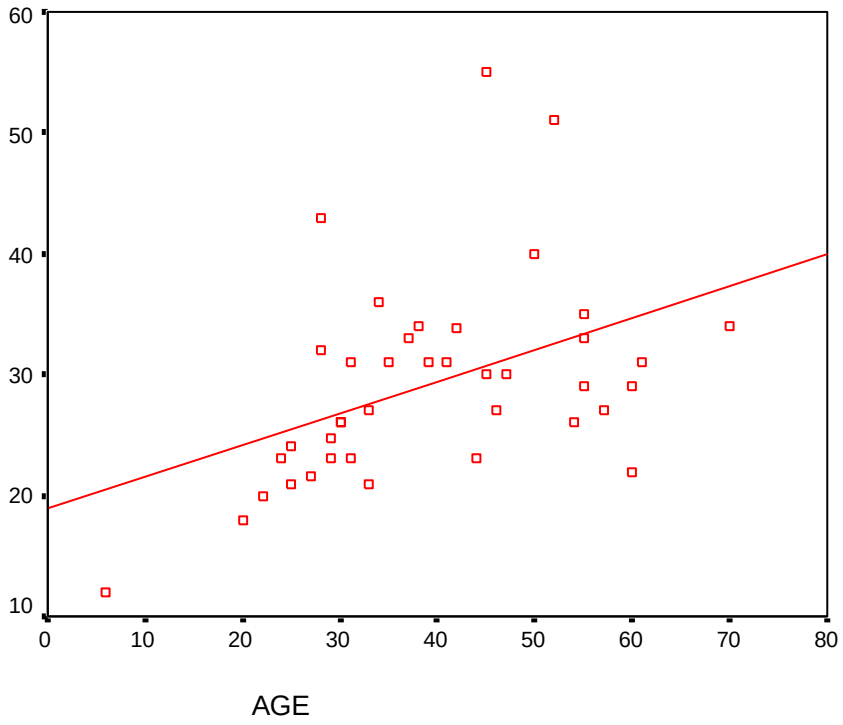


Figure (4-3): Show correlation between age and BMI (r= 0.442, *P.value* = 0.004).

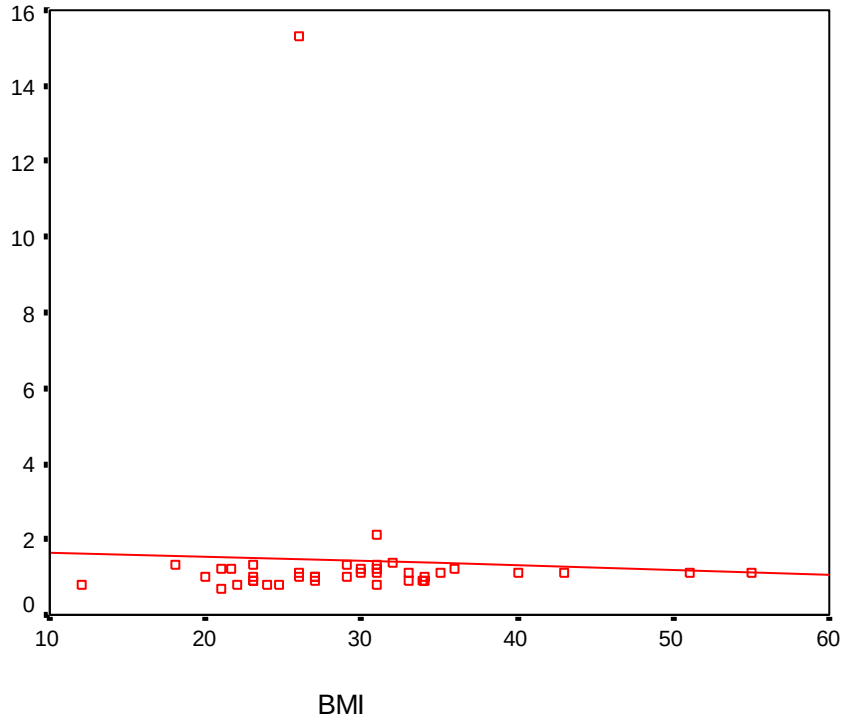


Figure (4-4): Show correlation between creatinine concentration in pre cupping and BMI (r= -0.043, *P.value* = 0.790).

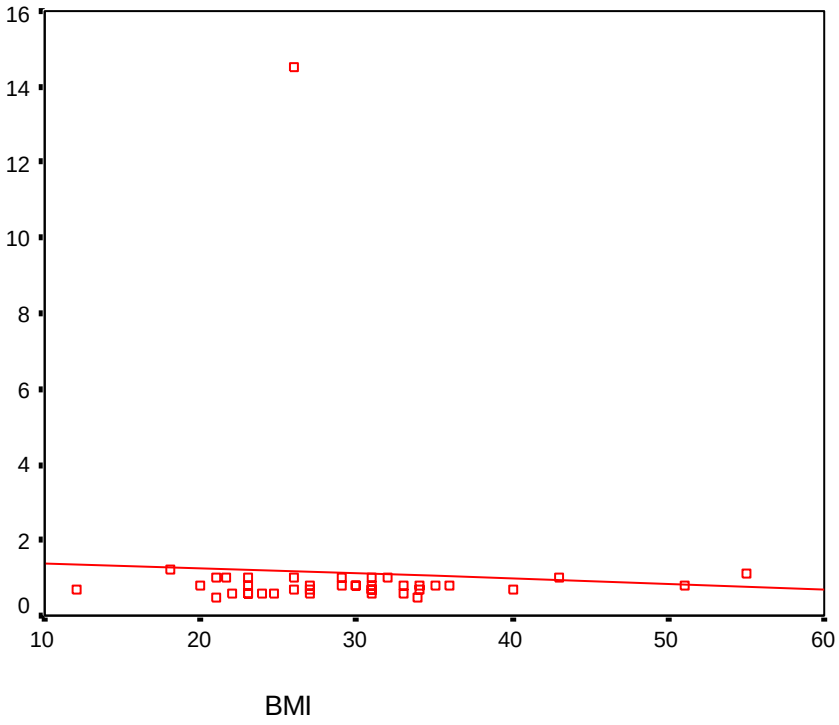


Figure (4-5): Show correlation between creatinine concentration in post cupping and BMI (r= -0.051, *P.value* = 0.756).

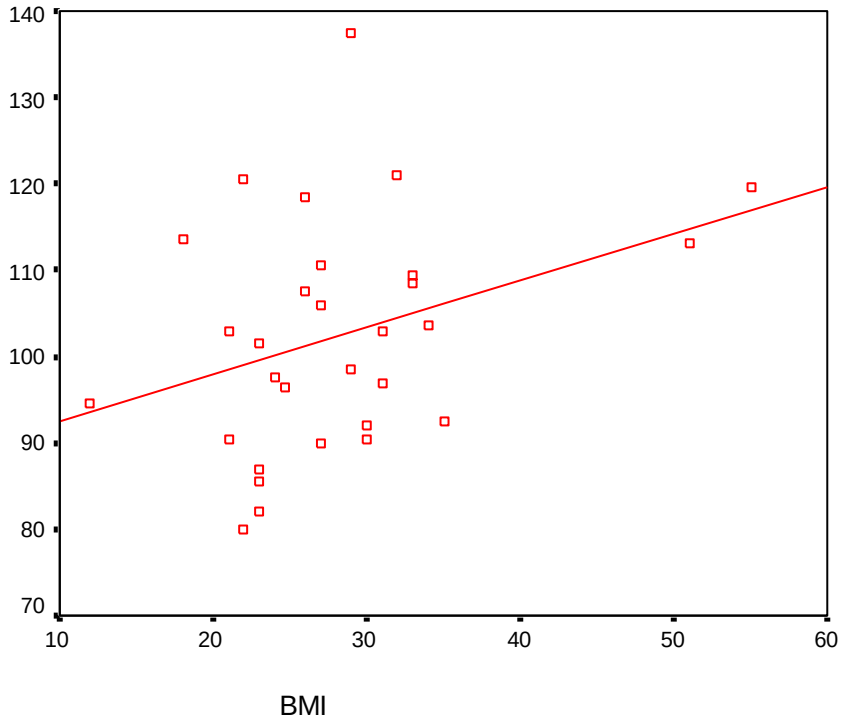


Figure (4-6): Show correlation between blood pressure in pre cupping and BMI (r= 0.347, *P.value* = 0.060).

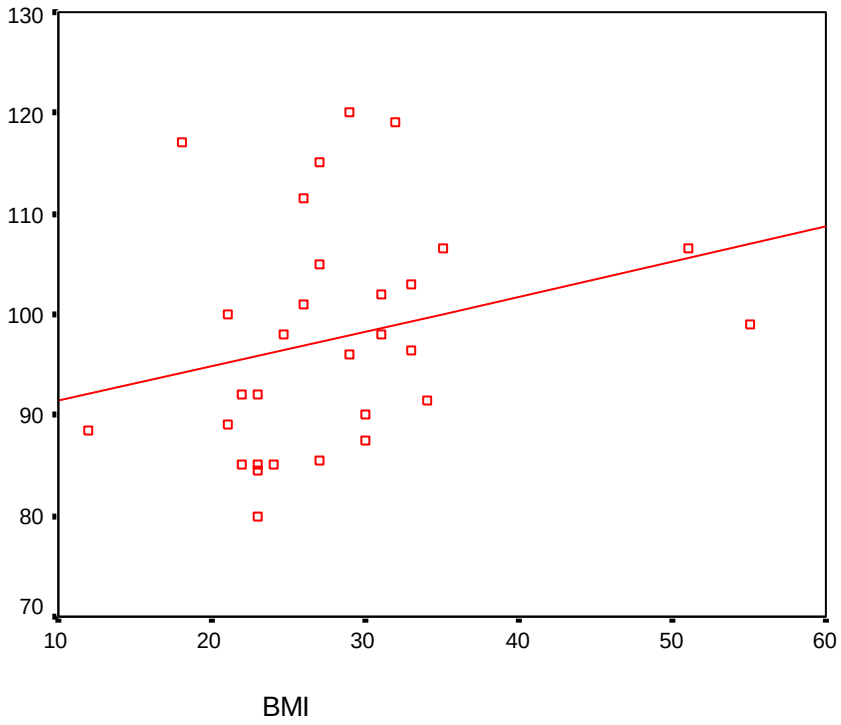


Figure (4-7): Show correlation between blood pressure in post cupping and BMI (r= 0.262, *P.value* = 0.161).

Chapter Five

Discussion, Conclusion

Recommendations

5. Discussion

At the time of improved public health service in Sudan, there was a marked increase in the number of patients referring to blood cupping, and the majority of them were young and educated patients but suffering from diseases with pain, long duration symptoms and non responsiveness to chemotherapy was the main motivator to turn to blood cupping. In this study, the samples were taken randomly and the patients aged from (20 to 70 years).

There was decrease in plasma creatinine level after hijama hence this change indicate that there have been improvement in renal function in patients after hijama, since patients with CRF are unable to maintain normal levels of creatinine. The physiological adverse effects of dialysis e.g. severe fatigue, weakness, insomnia and anorexia. However patients undergoing hijama reported reduction in all above mentioned physiological adverse effects and felt energetic, which helped in improving confidence and social relationship. (Muhammad et al., 2015)

The result of this study showed that there was a significant decrease in plasma creatinine after blood cupping (p.value =0.000) which is similer to the results were observed by (Fairouz, 2010; Bilal et al., 2011; Muhammad et al., 2015). Plasma uric acid also was significantly decreased after blood cupping (p.value =0.000) which is similer to the results were observed by (Fairouz, 2010). This shows that hijamah can at least improve symptoms and is especially useful when done over the site of pain in the case of gout.

Elevated BP and hypertension are major risk factors for CVDs, especially CHD, stroke and heart failure, as well as renal failure (WHO, 2013). Arecent meta-analysis that included two randomized controlled trials using wet and dry cupping in the treatment of hypertension has reported that using cupping therapy is beneficial in controlling and lowering BP. (Lee, 2010) In the absence of other

treatments of hypertension, blood cupping technique is said to promote blood circulation, remove stasis and could sometimes have had a beneficial effect in temporarily reducing blood pressure by a reduction in blood volume, including the fluid overload of heart failure(Fairouz, 2010). In this study blood pressure was significantly decreased after blood cupping (p.value =0.008) which is similer to the results were observed by (Fairouz, 2010) (Bilal et al., 2011).

The result showed that, there was a significant positive correlation between age and BMI (r=0.442 ,p.value=0.004) .

Study showed that there is a significant positive correlation between creatinine and uric acid in pre cupping (r=0.379 , p. value 0.016) and a significant positive correlation between creatinine and uric acid in post cupping (r=0.399, p.value =0.011) which is similer to the results was observed by (Fairouz, 2010). These findings given an accurate renal function assessment which are important in the diagnosis and treatment of kidney diseases, adjustment of drug dosages and decision-making regarding when to initiate renal replacement therapy. Studies from the general population suggest that obesity also may be harmful to the kidney in individuals without hypertension, diabetes or pre existing renal disease. **(**Fairouz, 2010).

Also study showed there is a significant positive correlation between BMI and blood preassure in pre cupping (r=0.347 , p.value =0.060) and insignificant weak positive correlation in post cupping (r=0.262 , p.value =0.161). Also the result showed that, there was no correlations were found between BMI with creatinine in pre and post cupping

(r=-0.043 , p.value 0.790) (r=-0.051 p.value =0.790) respectively and no correlations were found between BMI with uric acid in pre and post cupping(r=0.133 , p.value 0.415) (r=-0.151 , p.value =0.353) respectively .

Conclusion

The findings of this study indicate that blood cupping could decrease creatinine ,uric acid, blood pressure and may contribute to hypertension treatment. From the whole previous points we conclude that these data suggest that blood cupping is an adequate and safe technique, might be associated with decreased risk of cardiovascular disease, and enhanced and improved kidney function test.

Recommendations

1-More test can be perfomed such as(urea , Na , K) to evaluat the renal function.
2-Measurment of blood preassure in intervals.
3-Taking samples in different intervals after cupping to get more valuble results.
4.Taking patients diagnosed with gout to show the benefit of cupping on uric acid.

References

References

Ahmed, S.M., Madbouly N.H., Maklad, S.S., Abu-Shady, E.A.(2004). Immunomodulatory effects of blood letting cupping therapy in patients with rheumatoid arthritis. The EgyptianJournalof Immunology/Egyptian Association of Immunologists.;12(2):39-51.

Ahmadi, A., Schwebel, D.C., Rzaei, M. (2008). The Efficacy of Wet-Cupping in the treatment of tension and migraine headache .The Am.J .Chinese Medicine , 36(1),37-44.

Akhtar, J., Siddiqui, M.K.(2007). Utility of cupping therapy hijamat in unani medicine. India n J . Traditional knowledge , 13 (1) ,79-82.

Al-Rubaye, K.Q.(2012). The clinical and histological skin changes after the cupping therapy (Al- Hijamah). J Turk Acad Dermatol;6(1):1261a1.

AL-Shamma, Y.M., Abdil Razzaq, A.(2009). Al- Hijamah cupping therapy. Kufa Med J.;12(1).

Anees, S., Arafath, Y., Naaz, A., Khan, M.Q. (2015). Hijamah (Cupping therapy) as a preventive medicine-a retro-prospective analytical study. International Journal of AYUSH;4(2):88-100.

Baghdadi, I.H.(2005). Kitab-ul-mukhtarat-fil-tib. Translated by the Central Council for Research in Unani Medicine. New Delhi: Ministry of Health and Family Welfare, Govt. of India.: 277–278. Urdu

Baguanfa treatment. (2005)Cupping therapy ICTA; .Available: www. Cupping therapy.org/pages/applications.html .

Barham, D., Trinder, P.(1972). An improved colour reagent for the determination of blood glucose by oxidase system. Analyst; 27:142-145.

Bartels, H., Böhmer, M.(1971). Eine mikromethode zur kreatininbestimmung. Clin Chim Acta; 32: 81-85.

Bilal, M., Khan, R.A., Ahmed, A., Afroz, S. (2011). Partial evaluation of technique used in cupping therapy. *J. Basic Appl. Sci.*, **7**: 65-68.

Bondok, S. (2010). Cupping: The Great Missing Therapy, p. 136. Darussalam, Cairo.

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: Asystematic 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 review ofthe efficacy of cupping therapy. PLoS One.;7(2):e31793.

Cao, H., Zhu, C., Liu, J.(2010). Wet cupping therapy for treatment of herpes zoster: Asystematic review of randomized controlled trials. Alternative Therapies in Health and Medicine.;16(6):48.

Chen, P.F., Zheng, C.Y.(2010). Clinical observations of electroacupuncture and bleeding cupping therapy in treating postherpetic Neuralgia of Herpes Zoster [J]. Journal of Modern Clinical Medicine;1:026.

Chirali, Ilkay, Z.(2014). Traditional Chinese medicine cupping therapy. Elsevier HealthSciences.;11,14,127,129.

Chirali, I.Z .(1999). The cupping procedure. Traditional Chinese Medicine Cupping Therapy. London: Churchill Livingstone;. p. 73-86.

Cui, JI.N., Zhang, guangqi .(1989). A survey of thirty year clinical application of cupping, Journal of Traditional Chinese Medicine, 9 (3): 151-154,

Davis Company .(2007) Clinical Chemistry:A Laboratory Perspective. United States of America:203-204.

Duh, F.C., Chiu, Y.H.(2015). Vacuum cupping under various negative pressures: An experimental investigation. Vacuum.;2(7).

Duh, F.C., Yu, Z.Y., Chen, C.H.(2015). A study on avoiding burn injuries when treating patients with needle cupping. J. Multidiscip. Eng. Sci. Technol.;2(6): 1484-9.

El Sayed, S.M., Mahmoud, H.S., Nabo, M.MH.(2013). Medical and scientific bases of wet cupping therapy (Al-hijamah): in light of modern medicine and prophetic medicine. Altern Integ Med 2: 1-16

Evans, R.W., Mannien, D.C., Garrison, L.P., Hart, L.G., Blagg, C.R., Gutman, R.A., Hull, A.R., Lowrie, E.G.(1985). The quality of life of patients with end-stage renal disease. New Engl. J. Med., 312: 553-559

Fabiny, D.L., Ertingshausen, G.(1971). Automated reaction-rate method for determination of serum creatinine with CentrifiChem. Clin Chem; 17: 696-700.

Fairouz, K., Alshowafi .(2010). Effect of Blood Cupping on Some Biochemical Parameter. Medicaljournalofcairouniversity. Vol. 78, No. 1, June: 311-315.

Feroz,osman .(2013). Islamic Cupping & Hijamah: A Complete Guide. 11 Mandrill Street Lenasia 1827, South Africa.

Fossati, P., Prencipe, L., Berti, G.(1980). Use of 3,5-dichloro-2-hydroxy benzene sulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clin Chem; 26:227-231.

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.

Ibn Sina, Institute of Tibb .(2007). Regimental Therapies in Tibb, pp.17- 20. Johannesburg, South Africa.

Jan, Koolman., Klaus-Heinrich Roehm. (2005). Color Atlas of Biochemistry.2nd Ed. Thieme Stuttgart · New York.

Jiang, T., Qi, X.Z., Wu, B. (2004). Therapeutic effect of massage cupping ultrahort and acupuncture on cure facial-neuritis. Health J. Anhui Tech. Coll.;3(5):46-7.

Jiang, Zhen.Yal., He, ling.nal., Li, Chang.du. (2004). Treatment of 48 cases of menopause syndrome by moving Cupping therapy along the meridians.J.Acupuncture and Tuina Sci., 2(4), 37-38

Kaleem, U., Younis, A., Wali, M. (2007). An investigation into the effect of cupping therapy as atreatment for anterior knee pain and its potential role in health promotion .The Internet J .Alternative Med.,4(1)

Kaplan, N.M.(2002). Kaplan's clinical hypertension. 8th ed. Philadelphia, Lippincott Williams and Wilkins.

Kim, J.I., Kim, T.H., Lee, M.S., Kang, J.W., Kim, K.H., Choi, J.Y., Kang, K.W., Kim, A.R., Shin, M.S., Jung, S.Y., Choi, S.M.(2011). Evaluation of wetcupping therapy for persistent non-specific low back pain: A randomised, waiting-list controlled, open-label, parallel-group pilot trial. Trials.;12(1):146.

Kim, J.I., Lee, M.S., Lee, D.H., Boddy, K., Ernst, E.(2011). Cupping for treating pain: A systematic review. Evidence-Based Complementary and Alternative Medicine.

Kim, T.H., Basargard, L., Kim, J.I., Lee, M.S. (2011). Mongolian traditional style blood-letting therapy: abrief introduction . Complement Ther Clin Pract 17:179-183.

Lacey, J.M., Tershakovec, A.M., Foster, G.D.(2003). Acupuncture for the treatment of obesity: Areview of the evidence. InternationalJournal 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, K.E., Rampp, T., Saha, F.J., Dobos, G.J., 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 randomised controlled pilot study. BMC Complementary and Alternative Medicine.;11(1):1.10. Granter R, AdDipRemMass B. Myofascial vacuum cupping. Inside this Issue: 17.

Lee, M.S., Choi, T.Y., Shin, B.C., Kim, J.I., Nam, S.S. (2010). Cupping for hypertension: a systematic review. Clin Exp Hypertens 32:423-425.

Lin, M.L., Wu, H.C., Hsieh, Y.H., Su, C.T., Shih, Y.S., Lin, C.W., Wu, J.H.(2012). 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.

Lone, A.H., Ahmad, T., Anwar, M., Habib, S., Sofi, G., Imam, H.(2011). Leech therapy—a holistic approach of treatment in Unani(Greeko-Arab) medicine. Anc Sci Life; 31(1): 31–35.

Manz ,Hedwig. (2009). The Art of Cupping, pp. 3-4. Thieme, Stuttgart, Germany.

Muhammad, Bilal., Rafeeq, Alam., Khan, Khurram, Danial. (2015). Hijama improves overall quality of life in chronic renal failure patients: A pilot studyPak. J. Pharm. Sci., Vol.28 No.5, September , pp.1731-1735

Nessar, Ahmed.(ed) .(2011). clinical biochemistry.oxford: united states :71-113

Peake, M., Whiting, M. (2006). Measurement of serum creatinine-current status and future goals. Clin Biochem;27:173-184.

Ray, K., Suzanne, M. (2012). Biomedical Sciences .essential laboratory Medicine. John Wiley & Sons, Ltd . : 58-235.

Rashid, Bhikha., Ashraf, Dockrat. (2015). Medicine of the Prophet Tibb al-Nabawī your guide to healthy living . South Africa : Ibn Sina Institute of Tibb.

Shaban, T. (Encyclopedia– Create space publishing - ISBN-13: 978-1494780517;47-55.2013) Cupping therapy .

Teut, M., Kaiser, S., Ortiz, M., Roll, S., Binting, S., Willich, S.N., 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, L.M., Lee, H.P., Lu, C. (2006). Cupping: From a biomechanical perspective. Journal of Biomechanics.;39(12):2183-93.

Weber, J.A., Van, Zanten, A.P.(1991). Interferences in current methods for measurements of creatinine. Clin Chem; 37: 695-700.

Wei, Z . (2005). Treatment of hyperlipidemia by acupuncture and cupping method on back-shu point .J. acupuncture and Tuina Sci., 3(1), 14-15.

William, J., Marshall .(2012). Clinical Chemistry(7nd) Ed. London, UK.

Winkes, M.B., Hoogeveen, A.R., Houterman, S., Giesberts, A., Wijn, P.F., aScheltinga, M.R. (2012). Compartment pressure curves predict surgical outcome in chronic deep posterior compartment syndrome. The American Journal of Sports Medicine.; 40(8):1899-905. 0363546512449324.

World health organization. (2013). A global brief on hypertension Silent killer. Global Public Health Crisis 82.

World health organization.(2005). Clinical guidelines for the management of hypertension. Regional office for the eastern Mediterranean, Cairo.p:13

Wright, SM., Finical, J .(2000). Beyond leeches : Therapeutic phlebotomy today. Am. J .Nurs., 100 (55,56),58-61.

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

Yoo, S.S., Tausk, F.(2004). cupping:East meets West .International Journal of Dermatology,43(9),pp.664-665.

Appendix

Appendix I

Questionnaire

Sudan University of science and Technology

College of Graduate Studies

The Effect of Wet Blood Cupping on Plasma Creatinine and Uric Acid levels

Name :…….

Patient Number ( ) Phone Number ( )

Weight ( ) Hight ( )

Age ( ) BMI ( )

Pre blood preassure ( ) Post blood preassure ( )

Reason of hijama :……

Presence of chronic diseases : Yes ( ) No ( )

If yes : type of disease :……

Duration ( )

Treatment :……...

Laboratory investigations:-

Test name	Concentration
Creatinine	Mmol/L
Uric acid	Mmol/L

Appendix II

COD 11902 2 x 50 mL	COD 11502 4 x 50 mL	COD 11542 1 x 1 L
STORE AT 2-30ºC		
Reagents for measurement of creatinine concentration Only for in vitro use in the clinical laboratory		

CREATININE

CE

BioSystems

CREATININE
JAFFE

PRINCIPLE OF THE METHOD

Creatinine in the sample reacts with picrate in alkaline medium forming a coloured complex (Jaffé method). The complex formation rate is measured in a short period to avoid interferences[1-4]. Serum and plasma samples contain proteins that react in a non specific way; nevertheless, the results can be corrected subtracting a fixed value. The use of this correction is known as the Jaffé method compensated[5,6].

CONTENTS

	COD 11902	COD 11502	COD 11542
A. Reagent	1 x 50 mL	2 x 50 mL	1 x 500 mL
B. Reagent	1 x 50 mL	2 x 50 mL	1 x 500 mL
S. Standard	1 x 3 mL	1 x 5 mL	1 x 5 mL

COMPOSITION

A. Reagent: Sodium hydroxide 0.4 mol/L, detergent.

WARNING: H315: Causes skin irritation. H319: Causes serious eye irritation. P280: Wear protective gloves/protective clothing/eye protection/face protection. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P332+P313: If skin irritation occurs: Get medical advice/attention.

B. Reagent: Picric acid 25 mmol/L.

S. Glucose/Urea/Creatinine Standard: Glucose 100 mg/dL, urea 50 mg/dL, creatinine 2 mg/dL (177 µmol/L). Aqueous primary standard.

For further warnings and precautions, see the product safety data sheet (SDS).

STORAGE

Store at 2-30ºC.

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

Indications of deterioration:

- Reagents: Presence of particulate material, turbidity, absorbance of the blank over 0.350 at 500 nm (1 cm cuvette).
- Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Standard (S) is provided ready to use.

Working Reagent: Mix equal volumes of Reagent A and Reagent B. Mix thoroughly. Stable for 1 month at 2-8ºC.

ADDITIONAL EQUIPMENT

- Thermostatic water bath at 37ºC.
- Analyzer, spectrophotometer or photometer able to read at 500 ± 20 nm.

SAMPLES

Serum, plasma or urine collected by standard procedures. Dilute fresh urine 1/50 with distilled water before measurement. Heparin, EDTA, oxalate and fluoride may be used as anticoagulants.

Creatinine in samples is stable for 24 hours at 2-8ºC.

PROCEDURE

1. Bring the Working Reagent and the photometer to 37ºC.
2. Pipette into a cuvette (Note 1):

Working Reagent	1.0 mL
Standard (S) or Sample	0.1 mL

3. Mix and insert cuvette into the photometer. Start stopwatch.
4. Record the absorbance at 500 nm after 30 seconds (A_1) and after 90 seconds (A_2).

CALCULATIONS

The creatinine concentration in the sample is calculated using the following general formula (Note 2):

$$\frac{(A_2 - A_1)_{sample}}{(A_2 - A_1)_{standard}} \times C_{standard} \times \text{Sample dilution factor} - \text{Corrective Factor}^{5,6} = C_{sample}$$

If the Creatinine Standard provided has been used to calibrate (Note 3):

	Serum and plasma		Urine
	Jaffé non compensated	Jaffé compensated	
$\frac{(A_2 - A_1)_{sample}}{(A_2 - A_1)_{standard}}$	x 2 = mg/dL x 177 = µmol/L	x 2 - 0.37 = mg/dL x 177 - 33 = µmol/L	x 100 = mg/dL x 8840 = µmol/L

REFERENCE VALUES

Serum and plasma[7-9]:

Method	Jaffé non compensated	Jaffé compensated
Men Women	0.8 - 1.3 mg/dL = 63 - 115 µmol/L 0.6 - 1.1 mg/dL = 53 - 97 µmol/L	0.7 - 1.2 mg/dL = 62 - 106 µmol/L 0.5 - 0.9 mg/dL = 44 - 80 µmol/L

Urine[7]:

Men: 14 - 26 mg/kg/24-h = 124 - 230 µ mol/kg/24-h
Women: 11 - 20 mg/kg/24-h = 97 - 177 µmol/kg/24-h

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 10005, 10009 and 10042) and II (cod. 10007, 10010 and 10043) and the Biochemistry Control Urine (cod. 10054) to verify the performance of the measurement procedure.

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

METROLOGICAL CHARACTERISTICS

- Detection limit: 0.03 mg/dL creatinine = 2.65 µmol/L creatinine.
- Linearity limit: 20 mg/dL = 1768 µmol/L creatinine. For higher values dilute sample 1/2 with distilled water and repeat measurement.
- Repeatibility (within run):

Mean concentration	CV	n
1.1 mg/dL = 100 µmol/L	2.8 %	20
5.5 mg/dL = 490 µmol/L	1.3 %	20

- Reproducibility (run to run):

Mean concentration	CV	n
1.1 mg/dL = 100 µmol/L	2.8 %	20
5.5 mg/dL = 490 µmol/L	2.8 %	20

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 2). Details of the comparison experiments are available on request.
- Interferences: Hemoglobin (10 g/L), bilirubin (10 mg/dL), protein and ketonic bodies do not interfere. Lipemia (triglycerides = 2 g/L) may interfere. High concentration of reducing compounds may interfere. Other drugs and substances may interfere[7].

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

DIAGNOSTIC CHARACTERISTICS

Creatinine is a catabolic end product of creatine (or phosphocreatine). The amount produced each day is related to the muscle mass. Creatinine is freely filtered by the glomerulus (small amounts are reabsorbed and are also secreted by the renal tubules).

Creatinine measurement is used almost exclusively in the assessment of kidney function (impaired renal perfusion, loss of functioning nephrons) and in the monitoring renal dialysis[4,9].

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

NOTES

1. These reagents may be used in several automatic analyzers. Instructions for many of them are available on request.
2. For measurement in serum or plasma with the Jaffé method compensated, introduce the corrective value for the reaction of nonspecific proteins as a constant factor subtracted from the concentration value obtained[5,6].
3. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 10011 and 10044).

BIBLIOGRAPHY

1. Bartels H, Böhmer M. Eine mikromethode zur kreatininbestimmung. Clin Chim Acta 1971; 32: 81-85.
2. Fabiny DL, Ertingshausen G. Automated reaction-rate method for determination of serum creatinine with CentriChem. Clin Chem 1971; 17: 696-700.
3. Mazzachi BC, Peake MJ, Ehrhardt V. Reference range and method comparison studies for enzymatic and Jaffé creatinine assays in plasma and serum and early morning urine. Clin Lab 2000;46:53-65.
4. Tietz: Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co. 2006.
5. Weber JA, Van Zanten AP. Interferences in current methods for measurements of creatinine. Clin Chem 1991; 37: 695-700.
6. Peake M, Whiting M. Measurement of serum creatinine-current status and future goals. Clin Biochem 2006;27:173-184.
7. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press; 2000.
8. Friedman and Young. Effects of disease on clinical laboratory tests, 4th ed. AACC Press; 2001.

Appendix III

COD 11521 1 x 50 mL	COD 11521 1 x 200 mL	COD 11522 1 x 500 mL	COD 11540 1 x 1 L
STORE AT 2-8ºC			
Reagents for measurement of uric acid concentration Only for *in vitro* use in the clinical laboratory			

URIC A CID

CE

BioSystems

URIC A CID
URICASE/PEROXIDASE

PRINCIPLE OF THE METHOD

Uric acid in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry [1].

$$\text{Uric acid} + O_2 + 2\,H_2O \xrightarrow{\text{uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$2\,H_2O_2 + 4\text{-Aminoantipyrine} + DCPS \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4\,H_2O$$

CONTENTS

	COD 11521	COD 11521	COD 11522	COD 11540
A. Reagent B. Standard	1 x 50 mL 1 x 5 mL	1 x 200 mL 1 x 5 mL	1 x 500 mL 1 x 5 mL	1 x 1 L 1 x 5 mL

COMPOSITION

A. Reagent: Phosphate 100 mmol/L, detergent 1.5 g/L, dichlorophenolsulfonate 4 mmol/L, uricase > 0.12 U/mL, ascorbate oxidase > 5 U/mL, peroxidase > 1 U/mL, 4-aminoantipyrine 0.5 mmol/L, pH 7.8.

B. Uric Acid Standard: Uric acid 6 mg/dL (357 µmol/L). Aqueous primary standard.

STORAGE

Store at 2-8ºC.
Reagent and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.
Indications of deterioration:

- Reagent: Presence of particulate material, turbidity, absorbance of the blank over 0.200 at 520 nm (1 cm cuvette).
- Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Reagent and Standard are provided ready to use.

ADDITIONAL EQUIPMENT

- Thermostatic water bath at 37ºC.
- Analyzer, spectrophotometer or photometer able to read at 520 ± 10 nm.

SAMPLES

Serum, plasma or urine collected by standard procedures. Dilute urine 1/10 with distilled water before measurement.
Uric acid in serum or plasma is stable for 7 days at 2-8ºC. Heparin, EDTA, oxalate and fluoride may be used as anticoagulants.
Uric acid in urine is stable for 4 days at room temperature if pH is adjusted to > 8 with NaOH. Do not refrigerate.

PROCEDURE

1. Bring the Reagent to room temperature.
2. Pipette into labelled test tubes: (Note 1)

	Blank	Standard	Sample
Distilled water	25 µL	—	—
Uric Acid Standard (B)	—	25 µL	—
Sample	—	—	25 µL
Reagent (A)	1.0 mL	1.0 mL	1.0 mL

3. Mix thoroughly and incubate the tubes for 10 minutes at room temperature (18-25ºC) or for 5 minutes at 37ºC.
4. Measure the absorbance (A) of the Standard and the Sample at 520 nm against the Blank. The colour is stable for at least 30 minutes.

CALCULATIONS

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

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} \times \text{Sample dilution factor} = C_{Sample}$$

If the Uric Acid Standard provided has been used to calibrate (Note 2):

	Serum and plasma	Urine
$\frac{A_{Sample}}{A_{Standard}}$	x 6 = mg/dL uric acid	x 60 = mg/dL uric acid
	x 357 = µmol/L uric acid	x 5570 = µmol/L uric acid

REFERENCE VALUES

Serum and plasma[2]:

Men: 3.5-7.2 mg/dL = 210-430 µmol/L
Women: 2.6-6.0 mg/dL = 150-360 µmol/L

Urine[2]:

250-750 mg/24 h = 1.5-4.5 mmol/24 h

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18006 and 18042), level II (cod. 18007, 18010 and 18043) and the Biochemistry Control Urine (cod. 18056) to verify the performance of the measurement procedure.
Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

- Detection limit: 0.02 mg/dL = 1.19 µmol/L.
- Linearity limit: 25 mg/dL = 1485 µmol/L. For higher values dilute sample 1/5 with distilled water and repeat measurement.
- Repeatability (within run):

Mean Concentration	CV	n
3.30 mg/dL = 196 µmol/L	3.4 %	20
8.20 mg/dL = 488 µmol/L	3.3 %	20

- Reproducibility (run to run):

Mean Concentration	CV	n
3.30 mg/dL = 196 µmol/L	2.1 %	20
8.20 mg/dL = 488 µmol/L	1.8 %	20

- Sensitivity: 33.3 mA·dL/mg = 0.56 mA·L/µ mol
- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 2). Details of the comparison experiments are available on request.
- Interferences: Hemoglobin (2 g/L), bilirubin (2.5 mg/dL) and lipemia interfere. Other drugs and substances may interfere[3].

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

DIAGNOSTIC CHARACTERISTICS

In humans, uric acid is the major product of the catabolism of the purine bases which are obtained partly from the diet and partly from *in vivo* synthesis.
Increased uric acid concentration in serum and urine may be attributable to an overproduction of urate (increased purine synthesis) or to a defective elimination of urate[3].
Hyperuricemia is commonly associated with gout, decreased renal function, dehydration, myeloproliferative disorders, and other conditions not well known[3,4].
Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

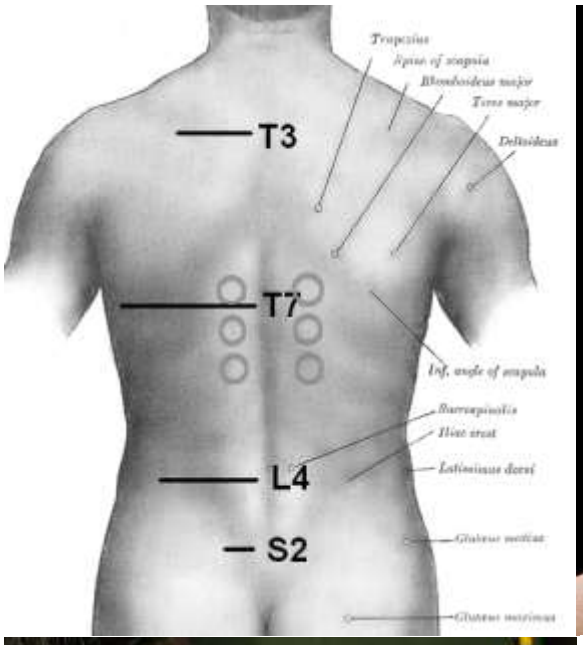
NOTES

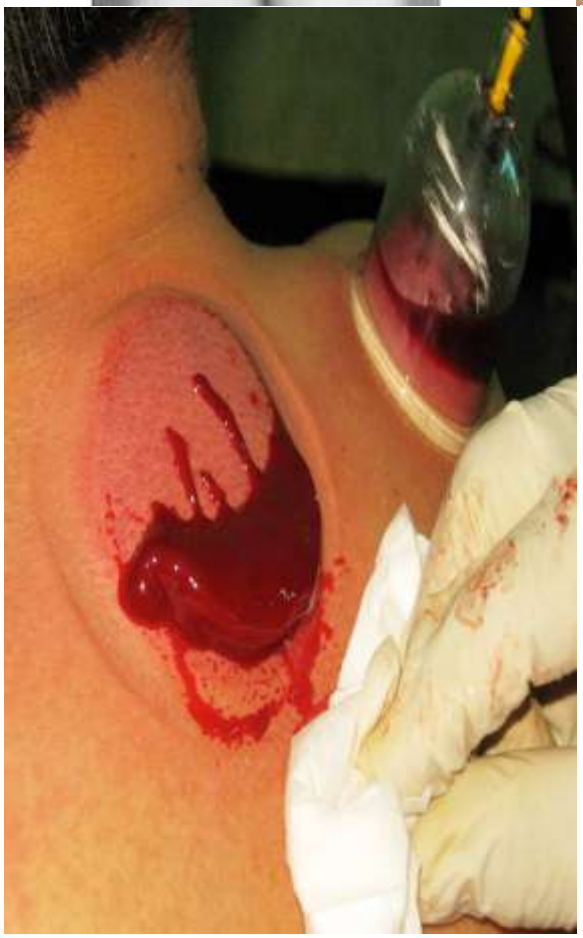
1. These reagents may be used in several automatic analysers. Specific instructions for application in many of them are available on request.
2. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analysers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

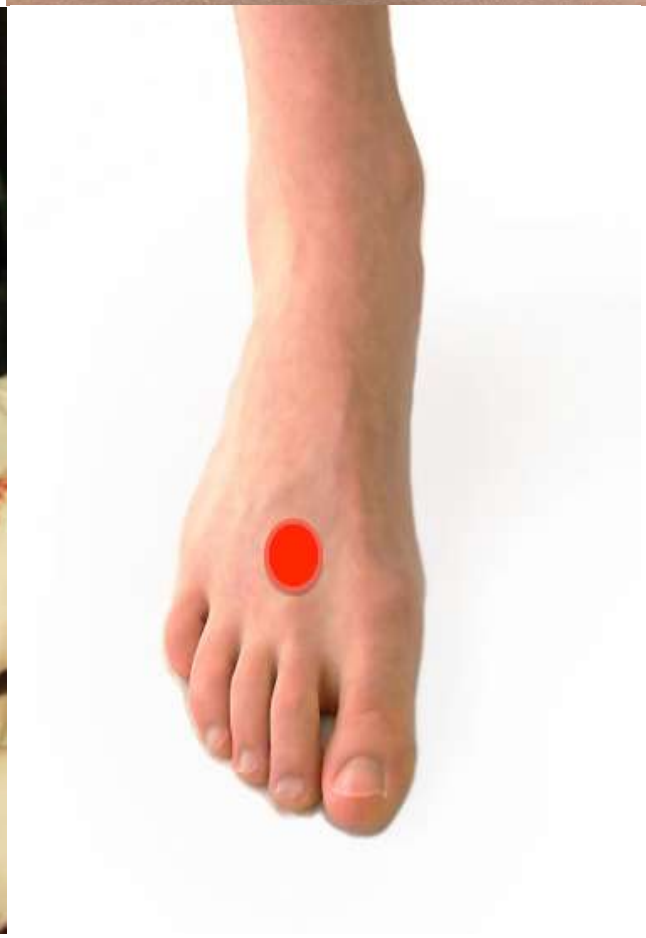
BIBLIOGRAPHY

1. Barham D, Trinder P. An improved colour reagent for the determination of blood glucose by oxidase system. Analyst 1972; 27:142-145.
2. Fossati P, Prencipe L, Berti G. Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clin Chem 1980; 26:227-231.
3. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co; 2006.
4. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press; 2000.
5. Friedman and Young. Effects of disease on clinical laboratory tests, 4th ed. AACC Press; 2001.

APPENDIX IV







Appendix V

Abu-Hurayrah reported “haddeeth” that reported by {Abu-Dawood, 3861 and Al-Bayhaqi, 00

Anas Ibn Maalk reported “haddeeth” that reported by {Saheeh Al-Bukhaaree ,5371}.