بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Assessmemt of Anticardiolipin Antibody and Urine Albumin Creatinine Ratio in Sudanese Pregnant Women with Preeclampsia

تقييم مستوى الأجسام المضادة للكارديوليبين ونسبة الألبيومين للكرياتينين في البول عند السودانيات الحوامل المصابات بمتلازمة ما قبل تسسم الحمل

A Dissertation Submitted in Partial Fulfillment for the Requirements of M.Sc. Degree in Medical Laboratory Science (clinical chemistry)

By:

Setana Suliman Mohammed Eltahir

B.Sc. in Clinical Chemistry, College of Medical Laboratory Science

Sudan University of Science and Technology, 2007

Supervised by:

Dr.Abdelgadir Ali Elmugadam

PhD Clinical Chemistry- Medical Laboratory Science

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الآيـــــة

بسم الله الرحمن الرحيم

(اللَّهُ نُورُ السَّمَاوَاتِ وَالْأَرْضِ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ الْمِصْبَاحُ فِي زُجَاجَةٍ الزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِنْ شَجَرَةٍ مُبَارَكَةٍ زَيْتُونَةٍ لَا شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارٌ نُورٌ عَلَى نُورٍ يَهْدِي اللَّهُ لِنُورِهِ مَنْ يَشَاءُ وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ)

صدق الله العظيم

سورة النور الآية (35)

Dedication

We are nothing but united fragments

Together we form the whole image & this time it was embodied in few people who made the journey worth walking

My beloved parents

Whose prayers, efforts & wishes are an inspiration

Who instilled in me the virtues of perseverance and commitment and relentlessly encouraged me to strive for excellence

Along with all hard working

People & respected teachers

Acknowledgement

First & Foremost, praises and thanks to the God,
The Almighty, for his showers of blessings throughout my research work to complete the research successfully

My sincere gratitude to my research supervisor

Dr. Abdelgadir Ali Elmugadam

For providing invaluable guidance throughout the research. It was a great privilege and honor to work and study under his guidance

Abstract

Hypertensive disorders of pregnancy is an umbrella term that includes preeclampsia (PE) which complicates up to 10% of pregnancies, is the cause of 9% to 26% of global maternal mortality, and represent a significant proportion of preterm delivery, and maternal and neonatal morbidity.

Is a potentially serious complication of pregnancy as suspected preeclampsia is the most frequent clinical presentation to obstetric unit. It is associated with severe complications such as seizures, stroke, and multiple organ failure.

The aim of this study is to assess anticardiolipin antibody and spot urinary albumin: creatinine ratio in pregnant women patients with preeclampsia.

This cross sectional study was conducted at Omdurman Hospital of Obstetrics and Gynecology on 80 Sudanese pregnant women according to selection criteria, and the study carried out in Omdurman Militarily Hospital in Khartoum State, on a total of 80 samples including 40 Preclamptic females as case group and 40 healthy pregnant females as controls. Serum anticardiolipin antibody (IgG aCL) levels was estimated using ELISA method, and urine albumin: creatinine ratio was estimated using Cobas Integra 400 plus automated analyzer. Data was analyzed by using the SPSS computer software program version 24.

This study showed that the levels of urine albumin: creatinine ratio was significantly increased with (P-value= 0.000); while there was insignificant decrease in levels of IgG anticardiolipin antibody (IgG aCL) with (P-value= 0.517) in preeclampsia patients compared to control group.

Insignificant negative correlation was observed between urine albumin creatinine ratio level and blood pressure (R=-0.002, P-value=0.989), also an association between IgG anticardiolipin antibody level and blood pressure was revealed insignificant negative correlation (R=-0.179, P-value =0.269).

There was insignificant weak positive correlation between levels of urinary albumin: creatinine ratio and IgG anticardiolipin antibody with value (R =0.295, P-value =0.064).

From the results and findings of this study, it is concluded that: The urinary levels of albumin: creatinine ratio are higher in preeclampsia patients, and the levels of IgG anticardiolipin antibody remained within the normal range in both case and control groups.

Negative correlation were found between blood pressure of preeclamptic pregnant women and their levels of urinary albumin: creatinine ratio and IgG anticardiolipin antibody.

المستخلص

إعتلال الضغط الدموي في الحمل مصطلح يشمل متلازمة ماقبل تسمم الحمل التي تتسبب بمضاعفات كما انها تمثل نسبة في الحمل بنسبة تصل الي 10% وسبب في وفاة الامهات عالميا بنسبة 9-26% معتبرة من حالات الولادة المبكرة و الحالات المرضية لدى الامهات والمواليد وتعتبر المتلازمة من مضاعفات الحمل التي يمكن ان تكون شديدة الخطورة حيث انها اكثر الحالات الاكلينيكية المترددة في وحدة التوليد وترتبط بمضاعفات حادة كنوبات مرضية والسكتة الدماغية وفشل عام في اعضاء الجسم

أجريت هذه الدراسة لتقييم مستوى الاجسام المضادة للكارديوليبين في الدم ونسبة الألبيومين للكرياتنين في البول وذلك لمعرفة إمكانية إستخدام هذه القياسات في التشخيص المبدئي للمتلازمة لدى النساء السودانيات في ولاية الخرطوم .

إشتملت الدراسة 80 إمرأة وفقا لعوامل تم تحديدها مسبقا ، و من ثم أخذت 40 عينة من النساء اللاتي يعانين من المتلازمة ليتم مقارنة النتائج مع 40 عينة أخرى لنساء حوامل من دون المتلازمة و اي امراض أخرى.تمت الدراسة خلال الفترة المحددة كما تم إجراء الاختبار بطرق تحليلة للقياس وأستخدم برنامج التحليل الاصدار24 (SPSS)الاحصائي.

أظهرت هذه الدراسة أن نسبة الألبيومين للكرياتينين في البول قد زادت بشكل ملحوظ مع قيمة (P=0.000)

في حين كان هنالك لا يوجد تغير في مستويات الأجسام المضادة للكارديوليبين بقيمة

(P=0.517)في المرضى الذين يعانون من المتلازمة مقارنة بفئة الأصحاء.

كما أثبتت الدراسة وجود ارتباط سلبي بين نسبة الألبيومين للكرياتنين في البول وضغط دم المرضى الذين يعانون من المتلازمة بقيمة

(R=-0.002, P=0.989)

وارتباط سلبي ايضا بين مستوى الاجسام المضادة للكارديوليبين وضغط دم المرضى بقيمة

(R=-0.179, P=0.269)

كما يوجد ارتباط ايجابي ضعيف بين نسبة الألبيومين للكرياتينين في البول ومستوى الأجسام المضادة للكارديوليبين بقيمة

(R=0.295, P=0.064)

خلصت الدراسة الى عدم اختلاف مستوى الاجسام المضادة للكارديوليبين بين الفئتين تحت الدراسة مما يقلل من إمكانية استخدام هذه الاجسام المضادة كأداة للتشخيص أو التنبؤ بحدوث المتلازمة خلال فترة الحمل.كما تدل زيادة نسبة الالبيومين للكرياتينين في البول لدى النساء اللاتي يعانين من المتلازمة على امكانية استخدام القياس كمؤشر جيد لزيادة بروتين البول وتدني وظيفة الكلى وبذلك التنبؤ بحدوث المتلازمة.

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List of Abbreviations

Abbreviations	Meaning
PE	Preeclampsia
aCL	Anticardiolpin antibody
aPLs	Antiphospholipid antibodies
APS	Antiphospholipid syndrome
ACR	Albumin: creatinine ratio
PCR	Protein: creatinine ratio
GFR	Glomerular filtration rate
CRP	C-reactive protein
BP	Blood pressure
BMI	Body mass index

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Chapter One

Introduction

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Chapter one

1-Introduction

1.1. Introduction

Hypertension is a common medical complication occurring in about (6-8%) of all pregnancies. Providers must be familiar with the diagnosis of pre-eclampsia, the hypertensive disorder associated with the high risk of adverse maternal and perinatal complications (Brown and Garovic ,2011), is an endothelial disease characterized by hypertension and commonly coinciding with proteinuria (Balen *et al.*, 2017). It is agreed that women with gestational hypertension (a new abrupt onset, a systolic blood pressure more than (140mm Hg) or a diastolic blood pressure more than (90mm Hg), on two occasions at least four hours apart in a previously normotensive patient, after 20weeks gestation and denovo appearance of proteinuria of more than 300 mg/24h urine collection or dipstick reading of 1+ is required for the diagnosis of pre-eclampsia (Sachan *et al.*, 2017).

Preeclampsia is known as the disease of multiple theories, among them genetics, immunology, circulatory factors, uterine vascular change and endothelial dysfunction. It is of utmost importance to highlight new diagnostic or predictive reliable tests and hence introduce and apply them in population with still family high maternal mortality rate (Fady *et al.*, 2014).

The kidney undergoes a number of change in internal structure and function during pregnancy. During normal pregnancy, the placenta mother produce large amount of hormones, such change in hormone levels can lead to change in angiotasis and increase water-sodium retention and the volume load, resulting in changes maternal hemodynamics and kidney structure and function. During normal pregnancy urinary albumin excretion increase significantly after 20 weeks to 12-19 mg (Qian yan *et al.,* 2016).

Renal plasma flow and Glomerular filtration rate increase by 40 to 65 and 50 to 85%, respectively, during normal pregnancy in women. Hyperfiltration is largely due to increased renal plasma flow, the latter attributable to profound reductions in both the renal afferent and efferent arteriolar resistances. Renal function is reduced in preeclamptic pregnancies, respectively, glomerular filtration rate (GFR) and renal plasma flow (RPF) were reduced by 32% and 24%. Currently, proteinuria is a hallmark of preeclampsia occurs secondary to

alterations in the size and/or charge selectivity of the glomerular filter, possible increases in glomerular capillary pressure, and compromise of proximal tubular -reabsorption. (Jeyabalan and Conrad, 2007). It has been found that the albumin excretion in urine correlates significantly to the albumin/creatinine ratio during pregnancy (Risberg *et al.*, 2004), and an alternative method for quantitative evaluation of proteinuria is the measurement of the albumin: creatinine ratio (ACR) in a spot urine sample (Huang *et al.*, 2012).

Antiphospholipid antibodies (aPLs), which include anticardiolipin antibodies (aCLs), are a heterogeneous group of autoantibodies associated with preeclampsia in patients with obstetric antiphospholipid syndrome (APS). Anticardiolipin antibodies share a common in vitro binding affinity for cardiolipin, the immunoglobulin isotypes may be IgG, IgM, and IgA.

Immunologic factors have long been considered to be key players in preeclampsia (Cecile *et al.*, 2016) and the exact mechanisms underlying thrombosis formation in APS are still unknown, but aPL can activate endothelial cells, platelets, monocytes, the complement system and coagulation factors, leading to impaired protein C activation and fibrinolysis and subsequent clot formation (Antovic *et al.*, 2018). Also the evidence of the association of anticadiolipin antiibodies with preeclampsia had been systematically reviewed using data sources (PubMed and LILACS were perused up to June 2009) and the conclusion found that moderate-to-high levels of (aCLs) are associated with preeclampsia (do prado et *al.*, 2010).

The majority of international organizations now recommend spot proteinuria tests in the assessment of suspected preeclampsia. ACR has been shown to be an accurate indicator of proteinuria in women with preeclampsia (.Elia *et al.*, 2017). International society for the study of hypertension in pregnancy had approved the spot urine (ACR) for detection of proteinuria (Sachan *et al.*, 2017).

Albumin excretion is considered to reflect glomerular damage and so a marker of systemic endothelial cell dysfunction (Elia *et al.*, 2017).Also the endothelial cell dysfunction that is characteristic of preeclampsia (immunologic effect of aCLs) may be partially due to an extreme activation of leukocytes in the maternal circulation (Cecile *et al.*, 2016).So this study is going on to detect an effective and practical early predictors of preeclampsia (spot urinary albumin:

creatinine ratio and anticardiolipin antibodies as markers of systemic endothelial cell dysfunction in preeclampsia).

Prediction of preeclampsia in the early stages of pregnancy can be very helpful in preventing the disorder or decreasing it is severity (Fady *et al.*, 2014), so new prognostic factors are needed in this area.

1.2. Rationale

Pre-eclampsia is a global health problem of increasing significance worldwide (9-26% of global maternal mortality) (Rosemary *et al.*, 2016). For the purpose of clinical management, acute rise in blood pressure in later half of pregnancy must be regard as pre-eclampsia, unfortunately, such diagnosis have been accepted uncritically in the selection of cases for clinical and laboratory studies of pre-eclampsia, with inevitably erroneous and contradictory conclusion about the disorder(Chesely, 1985), so there is a necessity for new tools for early detection, prevention, and management of the disorder have the potential to revolutionize practice in the coming years(Rosemary *et al.*, 2016).

The quantification of proteinuria is central to the investigation of hypertensive pregnant women which can be done by 24hr urine collection and protein estimation. This is the traditional method and consider as gold standard, but it has many drawbacks, it is cumbersome, suboptimal for facilitating rapid decision making, inconvenient and inaccurate (Sachan *et al.*, 2017). An estimate of proteinuria obtained from a random or spot sample would be simple, practical and appealing. Also the error to which the spot urine sample is susceptible that is, the potential mild variation in protein excretion- is likely to be far outweighed by the error associated with 24hr urine collection. Albumin: creatinine ratio like protein: creatinine ratio (PCR), it is measured using a random spot urine specimen, however it has increasing sensitivity as compared to (PCR) and too rapid as it can be performed using automated analyzer (Sanchez *et al.*, 2013). Obstetric antiphospholipid syndrome (APS) is now being recognized as distinct entity from vascular APS, preeclampsia occurs in up to 50% of pregnancies in women with the APS, precisely aCLs prevalence of 11-29%.

It is not well understood how antiphospholipid antibodies (aPLs), beyond their diagnostic and prognostic role, contribute to pregnancy manifestation (lppolito and Di simone, 2014), so aPLs may affect placental function through several mechanisms which are not mutually exclusive, to have clarified these

mechanisms has represented an important step allowing the introduction of new possible therapeutical possibilities able to abrogate the aPL pathogenic effect and provide elucidation for their association with pre-eclampsia.

Without clear national consensus guidelines on the origin of preeclampsia, the clinician is faced with management challenges and maternal and fetal risk-versus-benefit issues (Tammy and Martha, 2012).

In this study new diagnostic tests had been under evaluation ACR and aPL to address some of the inadequacies in current tests and obscurities in presentation, and as accompaniment to the rapid advances in understanding in recent decades.

1.3 Objectives

1.3.1 General objective

This study aimed to evaluate anticardiolipin antibody and urine albumin creatinine ratio in Sudanese pregnant women patients with preeclampsia.

1.3.2 Specific objectives

1-To measure anticardiolipin antibody (IgG) class and urinary spot albumin: creatinine ratio in healthy normotensive and pre-eclamptic pregnant women.

2-To compare the levels of anticardiolipin antibody (IgG) class and urinary spot albumin: creatinine ratio between the two included groups.

3-To correlate between age of the two compared groups, and the levels of IgG anticardiolipin antibody and urinary spot albumin: creatinine ratio respectively, and also to correlate between the levels of urinary spot albumin: creatinine ratio of the two compared groups, and levels of ambulatory blood pressure and anticardiolipin antibody (IgG) class respectively.

Chapter Two

Literature Review

Chapter Two
2-Literature Review

2.1.Changes during pregnancy

Pregnancy can change key of bodily processes and functions some of these changes occur to women metabolism. This is the way the body uses deity sugars, fats and proteins to provide the energy and building blocks needed to ensure the proper functioning of cells, tissues and organs (April *et al.*,2019).

Physiological changes occur in pregnancy to nurture the developing fetus and prepare the mother for labor and delivery. It is important to differentiate between normal physiological changes and disease pathology (Priya *et al*., 2016).

2.1.1.Changes in renal function

Hormonal changes during pregnancy allow for increased blood flow to the kidney and altered autoregulation such that glomerular filtration rate (GFR) increases significantly through reductions in net glomerular oncotic pressure and increased renal size (Wael and Richard, 2014).

As a consequence of renal vasodilation, the increase in plasma volume causes decreased oncotic pressure in the glomeruli with a subsequent rise in glomerular filtration rate (Priya *et al*., 2016). The mechanisms for maintenance of increased GFR change through the trimesters of pregnancy, continuing into postpartum period (Wael and Richard, 2014).

Preeclampsia is due to abnormal placentation, with shifts in angiogenic proteins and the renin- angiotensin-aldosterone system leading to endothelial injury and clinical manifestations of hypertension and organ dysfunction (Wael and Richard, 2014).

Renal dysfunction is a common complication of pregnancy. Important causes of pregnancy-specific renal dysfunction have been further studied, but much need to be learned (Wael and Richard, 2014).

2.1.2.Changes in immune system

The immune system changes during pregnancy, these changes contribute to the success of the pregnancy, and generally thought to be caused by the many hormonal changes that occur while women are with child. The mother immune system must be tightly regulated so that it does not reject the baby like I would

be a transplant organ. This is achieved by altering the numbers, location and activity of multiple subsets of maternal immune cells (April *et al.*, 2019).

The concept that pregnancy is associated with immune suppression has created a myth of pregnancy as a state of immunological weakness (Gil and Ingrid, 2010), so pregnancy may have an influence on autoimmune diseases improvement or worsening (Marie et al., 2016).

The relationship between autoimmunity and reproduction seems to be bidirectional. Accordingly, autoimmune diseases may selectively affect women in their reproductive years and conversely pregnancy may affect the expression of autoimmune diseases. Thus, autoimmunity may have an influence on pregnancy outcomes (Marie et al., 2016).

Antiphospholipid syndrome (APS) is associated with multiple autoantibodies, multiorgan involvement, more aggressive therapy, and increased impact on pregnancy outcome (Sara and Antonio, 2019). This disorder causes excessive clotting of the blood. It increases the mother risk of developing hypertension and preeclampsia and increases the baby risk of intrauterine growth retardation (IUGR), miscarriage, and stillbirth (Thais. 2019).

The generalization of pregnancy as a condition of immune suppression or increased risk is misleading and prevents the determination of adequate guidelines for treating pregnant women during pandemics (Gil and Ingrid, 2010).

2.1.3.Hypercoagulability in pregnancy

Hypercoagulability in pregnancy is the propensity of pregnant women to develop thrombosis (blood clots). Pregnancy itself is a factor of hypercoagulability (pregnancy induced hypercoagulability, as aphasiologically adaptive mechanism to prevent post-partum bleeding. However, when combined with an additional underling hypercoagulable states, the risk of thrombosis or embolism may become substantial (Gresele and Paolo, 2008).

The mechanisms of hypercoagulability include ability of pregnancy to change the plasma levels of many clotting factors, such as fibrinogen, which can rise to three times it is normal value. Thrombin levels increase. Protein S, an anticoagulant decreases, however the other major anticoagulants, protein C and antithrombin III, remain constant. Fibrinolysis is impaired by an increase in plasminogen activator inhibitor-2 (PAI-2) which synthesized from placenta.

Venous stasis may occur at the end of the first trimester, due enhanced compliance of the vessel walls by a hormonal effect (Gresele and Paolo, 2008).

Pregnancy can cause hypercoagulability by other factors as the prolonged bed rest, and pregnancy after the age of 35, and in itself it causes approximately a five-fold increased risk of deep venous thrombosis. Several pregnancy complications which cause substantial hypercoagulability such as preeclampsia. Also acquired. Hypercoagulability states as a pre-existing condition in pregnancy include antiphospholipid antibodies (Eichinger *et al.,* 2013).

2.2.Complications of pregnancy

The pregnant woman presents a diagnostic challenge as physiological, anatomical, and biochemical changes of pregnancy itself being the source of the problem. The pathologies occurring in early pregnancy are common but some may be life threating and it is therefore essential to promptly diagnose and treat complications to achieve the best fetal and maternal outcomes. (Anna *et al.*, 2014).

Some women experience health problems during pregnancy.These complications can involve the mother health, the fetus health, or both. Getting early and regular prenatal care can help decrease the risk for problems by enabling health care providers to diagnose, treat, or manage conditions before they become serious. Some common complications of pregnancy include, but are not limited to hypertension which increase the risk of preeclampsia (Leeman and Fontaine, 2008).

2.2.1.Toxemia in pregnancy

Toxemia is a multisystem disease, typically occurring in late pregnancy, with the usual clinical manifestations of hypertension, proteinuria, edema, and central nervous system irritability Toxemia accounts for approximately 70% of hypertension seen in pregnant women (Ferris and Francisco, 1982).

Because convulsions may occur with severe toxemia, the disease has been divided into eclampsia, a term used synonymously for a convulsive disorder until early in this century, and preeclampsia (PE) based on whether the seizure has occurred (Ferris and Francisco, 1982). PE risk to health not only in the immediate peripartum period- women who have suffered from preeclampsia are at increased risk of cardiovascular disease through life, and children born from pregnancies affected by preeclampsia are more likely to suffer from metabolic

syndrome, cardiovascular disease , and hypertension at earlier ages (Rosemary *et al*., 2016).

The first aim of treatment of toxemia should be it is prevention. Proper prenatal care reduces the incidence of the disease. (Ferris and Francisco, 1982).

2.2.2. Hypertension in pregnancy

Hypertensive disorders complicating pregnancy are common and form one of the deadly triad, along with hemorrhage and infection that contribute greatly to maternal morbidity and mortality (Fady *et al*., 2014).The prevalence of hypertension in reproductive-aged women is estimated to be 7.7% (Stephanie and Andrei, 2019).

In pregnancy there is alteration occur in the renin-angiotensin and prostaglandin system. Because prostaglandin is vasodilator and antagonist to vasoconstrictor effect of angiotensin, it is increased synthesis might be a factor in the fall in peripheral resistance and angiotensin resistance which occur during pregnancy (Ferris and Francisco, 1982).

2.2.2.1 Pathophysiology of hypertension

Any hypertensive disorder of pregnancy can result in preeclampsia. The underlying pathophysiology that upholds this transition to, or superposition of, preeclampsia is not well understood; however, it is thought to be related to a mechanism of reduced placental perfusion inducing systematic vascular endothelial dysfunction. This rises due to a less effective cytotrophoblastic invasion of the uterine spiral arteries .The resultant placental hypoxia induces a cascade of inflammatory events, disrupting the balance of angiogenic factors and inducing platelet aggregation, all of which result in endothelial dysfunction manifested clinically as the preeclampsia syndrome.

Angiogenic imbalances associated with the development of preeclampsia include decreased concentrations of angiogenic factors such as vascular endothelial growth factor (VEGF) and placental growth factor (PIGF) and increased concentration of their antagonist, the placental soluble fms-like tyrosine kinase 1(s FLt-1) (Stephanie and Andrei, 2019).

2.2.2.2.Toxemia and hypertension:

Hypertension may occur during pregnancy because of toxemia of pregnancy, a systematic disease unique to pregnant women in which hypertension is associated with renal disease manifested by proteinuria and reduction of glomerular filtration rate or hypertension may occur without evidence of renal involvement, in which case it is classified as essential or idiopathic hypertension(Ferris and Francisco , 1982).

Toxemia accounts for approximately 70% of hypertension seen in pregnant women. The mechanism of toxemia in pregnancy is not clear but, as in all types of human hypertension, peripheral resistance is increased, in addition sodium retention associated with toxemia may heightened peripheral resistance by arteriolar swelling. The cause of sodium retention in preeclampsia is most likely reduction in GFR. Preeclampsia formerly called toxemia, is when a pregnant woman has high blood pressure, protein in her urine, and swelling in her leg, feet, and hand (Ferris and Francisco, 1982).

2.3.Preeclampsia

Preeclampsia (PE) is one of hypertensive disorders, is defined as pregnancy-specific syndrome of reduced organ perfusion secondary to vasospasm and endothelial activation (Fady *et al*., 2014).

It remains one of the most complex challenges for perinatal clinicians and researchers. As a major cause of maternal and perinatal mortality and morbidity worldwide. This condition lacks an effective prevention strategy or curative treatment. Furthermore, the efforts to develop screening tests have been disappointing for potential use in clinical practice remain unclear, with no known biomarkers (Audibert, 2005).

The pathophysiology of pre-eclampsia remains poorly understood. Moreover, there is no reliable predictive test and no effective prophylactic therapy for this disease. Advances have, however, recently been made in our understanding of the genetics of pre-eclampsia. Prediction and prevention are intimately linked, and both problems will only be solved by further unravelling of the complex pathophysiology of pre-eclampsia (Higgins and Brennecke, 1998).

Currently, women at risk are identified on the basis of epidemiological and clinical risk factors, but the diagnostic criteria of pre-eclampsia remain unclear, with no known biomarker (Sibai *et al.*, 2005).

2.3.1.Preeclampsia epidemiology

Preeclampsia is a multisystem disorder that complicates 3%-8% of pregnancies in western countries and constitutes a major source of morbidity and mortality worldwide. Overall 10%-15% of maternal deaths are directly associated with preeclampsia. Some epidemiological findings support the hypothesis of genetic and immunological etiology (Jennifer *et al.,* 2011).

The risk of preeclampsia is 2-fold to 5-fold higher in pregnant with a maternal history of this disorder. Depending on ethnicity, the incidence of preeclampsia ranges from 3% to7% in healthy nulliparous and 1% to 3% for multiparas (Jennifer *et al.,* 2011).

There are important variations in the prevalence of preeclampsia between lower middle - income countries and high- income countries. For instance, preeclampsia is diagnosed in 3% of all pregnancies in United States (Wallis *et al.,* 2008), and 3.3%in New Zealand (Stone et al .1995) while in Colombia it is present in 9% and Haiti in 17% (Lopez-Jaramillo *et al.,* 2005).

Over 10 million women around the world develop preeclampsia annually, 76.000 pregnant women die each year from preeclampsia and related hypertensive disorders globally, and every 7 minutes loses her life due to these often preventable conditions. The impact hypertension disorders on global infant mortality is enormous. 500. 000 babies die from preeclampsia and other hypertension disorders annually, and over 2.5 million preterm birth are caused by preeclampsia each year **(**Kypros**.** 2017)

2.3.2.Preeclampsia Causes

There is no definite known cause of preeclampsia, though it is likely related to a number of factors. Preeclampsia results from impaired trophoblast differentiation and invasion in early pregnancy, which stimulates sustained oxidative stress and a systematic inflammatory response (English, Kenny, and McCarthy, 2015).

Studies have shown that alteration in the regulation and signaling of angiogenic pathway contributes to the inadequate cytotrophooblast invasion, resulting in preeclampsia. Endothelial dysfunction has been demonstrated as early as 22 weeks of gestation, and the levels of antiangiogenic factors starts rising as early as 17 weeks of gestation. It could be expected that microalbuminuria, a marker of endothelial dysfunction, might be apparent by this time (Vineet *et al.,* 2016).

2.3.3.Preeclampsia risk factors

High risk women include those with preexisting hypertension, chronic kidney disease, insulin-dependent diabetics, and women with previous early onset of preeclampsia. It is more common in prim gravida women .Age greater than 40 years of age and obesity increase risk, also previous history of preeclampsia and

Women suffering from medical condition such as antiphospholipid syndrome (English, Kenny, and McCarthy, 2015).

2.3.4.Preeclampsia diagnosis

Proteinuria is an important sign of preeclampsia, without which, the diagnosis is questionable (Fady *et al*., 2014).

Traditionally, the diagnosis of preeclampsia in a woman without preexisting hypertension or proteinuria required new onset of hypertension and proteinuria after 20 weeks gestation. In 2013, the American College of Obestetrians and Gynecologists Task Force on Hypertension in Pregnancy presented new diagnostic criteria for preeclampsia in the absence of proteinuria if other preeclampsia features are present such as systolic blood pressure more than 140 mmHg or diastolic blood pressure more than 90 mmHg, renal insufficiency with serum creatinine concentration more than 1.1 mg/dL or doubling concentration in the absence of other renal diseases, impaired liver function with elevation of blood concentrations of liver enzymes twice normal values, thrombocytopenia, pulmonary edema, and cerebral or visual symptoms (No author listed).

2.3.5.Preeclampsia prediction

Management of preeclampsia centers on early recognition and timely delivery to prevent serious morbidity and mortality. Despite recent advances in our understanding of the etiology of preeclampsia, there is still no clinically useful screening test.The diagnosis of preeclampsia in the community is by detection of persistent hypertension and proteinuria (English, Kenny, and McCarthy, 2015).

2.3.6. Management of preeclampsia

Without clear national consensus guidelines on the origin of preeclampsia, the clinician is faced with management challenges and maternal and fetus risk-versus-benefit issues. The management of preeclampsia has not changed significantly over time, effective management may be divided into three

categories; prevention of preeclampsia, early detection, and treatment (English, Kenny, and McCarthy, 2015).

Women considered to be at high risk of preeclampsia (such as those with chronic hypertension, coexisting renal disease, or antiphospholipid syndrome should be referred for pregnancy counseling to identify modifiable risk factors (English, Kenny, and McCarthy, 2015).

Although there is no established preventative therapy, there is still a potential benefit in being able to identify the women at risk, so that appropriate monitoring can be done. There is some evidence to support the prophylactic benefit of the early introduction of aspirin, calcium, and heparin in such high risk women (Vineet *et al.,* 2016).

2.4.Obesity and preeclampsia

Numerous studies have shown that obesity is associated with many complications during pregnancy, including preterm delivery and hypertensive complications (Yogev and Catalano, 2009).

The incidence of obesity is increasing at alarming rate. There is compelling evidence that obesity increases the risk of preeclampsia about 3-fold, and in developed countries is the leading attributable risk for the disorder (James *et al*., 2011). It is a major epidemic in developed countries that is now extending to developing countries (Misra *et al.*, 2008).

There are many common mechanisms that link obesity with a higher risk of developing preeclampsia (Spradley *et al.,* 2015). Pathophysiological features of preeclampsia including endothelial dysfunction, oxidative stress, and increased inflammatory activation, with this background, useful targets identified for the study of the role of obesity in preeclampsia (Kelesy, Leanne, and Jenny.2019).

It is not just total body fat but fat distribution and accrual that is important. Central obesity as a marker of visceral obesity presents much higher risk than peripheral obesity. Visceral fat is functionally different than subcutaneous fat. It produces more C-reactive protein (CRP) and inflammatory cytokines and contributes more to oxidative stress, so increased adiposity results in heightened state of systematic inflammation that can influence placental development, because adipose tissue is a rich source proinflammatory cytokines and complement proteins, which have been implicated in the pathogenesis of

preeclampsia by promoting the expression of antiangiogenic factors in the mother (Kelesy, Leanne, and Jenny.2019).

Also the endogenous inhibitor of nitric oxide synthase, asymmetric dimethyl arginine (ADMA), might be a convergence point for many of the potential mechanisms by which obesity increases preeclampsia risk (James *et al*., 2011).

A strong direct correlation was found between increasing body mass index (BMI) and the risk of developing preeclampsia and pregnancy induced hypertension (Fernandez Alba *et al.,* 2018).

Preeclampsia risk rose strikingly from a BMI of 15 to 30 Kg/m2. Compared with women with a BMI of 21, the adjusted risk of developing preeclampsia doubled for overweight mothers with a BMI of 26kg/m2, and nearly triple at a BMI of 30 Kg/m2 (Bodnar *et al.,* 2005). It is also evident that this relationship is not limited to obese and overweight women because increases in BMI in the normal range is also associated with an increased risk of preeclampsia (James *et al*., 2011).

2.5.Proteinuria and preeclampsia

Proteinuria is one of the essential criteria for the clinical definition of pre-eclampsia. It is part of the fundamental investigations performed by healthcare professionals in primary and secondary care to monitor disease severity and predict complications in women with pre-eclampsia. It occurs due to renal glomerular endotheliosis, a manifestation of widespread endothelial damage in preeclampsia. More recently, spot urine protein: creatinine ratio (PCR) has been used to provide an accurate quantification of 24-hour proteinuria (Shakila *et al*., 2009).

In a systematic review for estimation of proteinuria by urine protein: creatinine ratio as a predictor of complications of pre-eclampsia, electronic searches had been conducted in MEDLINE (1951 to 2007), EMBASE (1980 to 2007), the Cochrane Library (2007) and the MEDION database to identify relevant articles confirmed that measure of proteinuria is a poor predictor of either maternal or fetal complications in women with pre-eclampsia (Shakila *et al.,* 2009).

Same conclusion observed in a retrospective cohort study published in BJOG journal in 2005, All women with the diagnosis of proteinuric pre-eclampsia in the years 1998-2001 were studied in order to determine, whether a discriminant value of proteinuria at the time of diagnosis predicts the presence or absence of

subsequent adverse maternal and fetal outcomes by systematic quantitative review of test accuracy studies. This systematic review has shown that estimation of levels of proteinuria in women with pre-eclampsia is not a clinically useful and it is magnitude is a poor predictor of the major maternal and fetal complication test to predict fetal or maternal complications. Methodological deficiencies such as verification bias, differential use of reference standards and case-control design did not apply to the studies in the review, ensuring inclusion of studies of acceptable quality, however a significant limitation of this review is the heterogeneity noticed between individual studies with regards to population, definition of pre-eclampsia, method of performing the test, test thresholds, frequency of testing, interval between the test and outcome, and reference standards (Chan *et al.*, 2005).

Also in article in Pregnancy Hypertension journal a question of should the spot albumin-to-creatinine ratio (ACR) replace the spot protein-to-creatinine ratio (PCR) as the primary screening tool for proteinuria in pregnancy? , had been answered as that the ACR is not inferior to nor does it perform better than the PCR in screening for proteinuria in pregnancy.

Clinicians should use the test with which they are more familiar and may wish to assess local laboratory costs and methods in their selection (Cade et *al.*, 2015).

2.5.1.Urine albumin: creatinine ratio (ACR) as a marker of significant proteinuria in preeclampsia

Many reviews postulated the using of new tools in the diagnosis and this reviews present the current best practice in diagnosis and management of the disorder, these studies emphasized the accuracy of the predictive value of ACR in predicting complications in women with preeclampsia.

ACR is routinely used outside of pregnancy to detect proteinuria, and may be superior to protein: creatinine ratio (PCR), but have yet to be validated in pregnancy and preeclampsia (Rosemary *et al*., 2016).

To review the spot albumin: creatinine ratio as diagnostic tests for significant proteinuria in hypertensive pregnant women, a systematic review depend on Literature search (1980-2007) for articles of the spot albumin: creatinine ratio in hypertensive pregnancy, with 24 hour proteinuria as the comparator, had been published in 2008 and concluded in two studies that the diagnostic accuracy for

the spot albumin: creatinine ratio was excellent when compared with 24 hour proteinuria and is a reasonable "rule-out" test for detecting proteinuria of 0.3 g/day or more in hypertensive pregnancy, however Information on use of it is insufficient. One of the limitations of this review was inadequate reporting of completeness of 24 hour urine collection and its use as the traditional comparator for diagnosing proteinuria and this also noticed in previous cohort study of 2005.The strengths of review include focus on primarily hypertensive pregnant women and a favorable diagnostic test characteristics for a cut-off point of 30 mg/mmol, as recommended by international societies had been reported, also no significant heterogeneity in cut-off points was found between studies over a range of proteinuria in contrast to a previous retrospective cohort study of 2005 which shown a significant heterogeneity(Cote *et al.*, 2008).

A Prospective study carried out over a period of one year in the Department of Obstetrics and Gynecology on thirty preeclampsia women, and had been published in 2017 on Nigeria medicine journal, conclude an association of raised ACR values with severity of disease as well as with adverse fetomaternal outcome (strong correlation between urinary ACR levels and 24 h urinary proteins) was observed (Sachan *et al.*, 2017).

From December 2009 to February 2012 with analysis of demographic, clinical and biochemical data from two obstetric day assessment units in hospitals in Southeast Scotland, 717 pregnant women with pregnancies after 20 weeks gestation included in retrospective cohort study published in 2017, ACR had been shown to be an accurate indicator of proteinuria in women with preeclampsia & is an independent prognostic factor for maternal and neonatal adverse outcomes in suspected pre-eclampsia (Elia *et al.*, 2017).

Evaluation of spot urinary albumin–creatinine ratio as screening tool in prediction of pre-eclampsia in early pregnancy had been performed in prospective observational study and the conclusions were spot urinary ACR values are higher in asymptomatic women in early pregnancy, who developed pre-eclampsia later on when measured early in the second trimester and it can be used as a good screening tool for predicting pre-eclampsia in early pregnancy (Vineet *et al.*, 2016).

The maternal spot urine estimate of protein to creatinine ratio (PCR) shows promising diagnostic value for significant proteinuria in suspected preeclampsia. The existing evidence is not, however, sufficient to determine

how PCR should be used in practice, owing to the heterogeneity in test accuracy and prevalence across studies. Insufficient evidence is available on the use of albumin to creatinine ratio (ACR) in this area. Insufficient evidence exists for either test to predict adverse pregnancy outcome (Morris *et al*., 2012).

Using of albumin to creatinine ratio (ACR) in random urine samples as appropriate screening test for proteinuria or for the disease severity in hypertensive disorders with pregnancy needs still to be verified (Fady *et al*., 2014).

2.6.Association of anticardiolipin antibody with preeclampsia

It is widely believed that the IgG isotype is most strongly associated with thrombosis, and as it has great capability to cross the placenta, wherefore this study focused on this isotype.

The existing literatures supported a hypothesis of possible association between pre-eclampsia and anticardiolipin antibody were inconsistent, with varying severity of disease phenotype examined, differing (aPL) titer cutoffs used to define positive status, and an overwhelming lack of repeat confirmatory (aPL) testing. This calls into question the link between (aPLs) and pre-eclampsia, or at least makes it less well defined (Gibbins and Branch, 2014).

A cross-sectional study published in 1999, was conducted in 39 women with preeclampsia and normotensive pregnant women in the 3rd trimester of pregnancy and the findings shown that There were no significant differences in IgG anticardiolipin antibody (IgG aCL) between patients with preeclampsia and normotensive pregnant women, Serum aCL levels were similar in both (Abundis *et al.*, 1999).Same conclusion observed in comparative study published in 1997(D'Anna *et al.*, 1997).

It is unlikely that antiphospholipid antibodies (anticardiolipin antibodies) represent risk factors for preeclampsia among women with no previous preeclampsia and no histories of thrombosis or systemic autoimmune disease, this was observed in a prospective case-control study of 180 pregnant women with their first incidents of preeclampsia and no histories of thrombosis or systemic autoimmune diseases to assess the association between the occurrence first of preeclampsia and antiphospholipid antibodies (Dreyfus *et al.*, 2001).

Article presented at the Nineteenth Annual Meeting of the Presented at the Nineteenth Annual Meeting of the American Gynecological and Obstetrical

Society in 2001, connoted Testing for antiphospholipid antibodies during pregnancy is of little prognostic value in the assessment of the risk for recurrent preeclampsia among women with a history of preeclampsia (Branch *et al.,* 2001).

A case-control study published in 2018 sub served that a systematic review and meta-analysis revealed a heterogeneity among the studies; out of nine studies, four studies demonstrated that anticardiolipin antibodies increased the chance of preeclampsia but five studies reported there was no statistically significant associations. This heterogeneity might occur due to the difference in their case selection, clinical, demographic or epidemiological variances (Gibbins *et al.,* 2018).

The presence of antiphospholipid antibodies (aPLs) is considered a risk factor for pre-eclampsia, two meta-analysis and a number of case control and cohort studies have found association between pre-eclampsia and anticardiolipin antibodies (aCLs) (Gibbins and Branch, 2014).

In order to determine the incidence of anticardiolipin antibodies in women with preeclampsia, Sera from 100 women with preeclampsia and 100 normotensive pregnant women in the third trimester were assayed for anticardiolipin antibodies IgM antibodies to cardiolipin were positive in two patients (2%) while IgG antibodies to cardiolipin were positive in nine patients (9%). Only 3% of the control women were positive for antiphospholipid antibodies. Elevated levels of IgG or IgM antibodies to cardiolipin and were detected in 11/100 (11%) of women diagnosed with preeclampsia in the third trimester compared to only 3/100 (3%) positive in controls ($P < \text{or} = 0.05$). These findings suggest that antiphospholipid antibodies may play a pathogenic role in some women with preeclampsia. This study was published in 1996 on article in American journal of reproductive immunology (Allen *et al.,* 1996).

In Journal of hypertension in Pregnancy the assumed association was confirmed in prospective, observational study was conducted to conclude that the proportion of antiphospholipid antibodies was somewhat higher in preeclamptic than in normotensive pregnant (Schjetlein *et al.,* 1998).

Anticardiolipin antibody was found to be associated with adverse outcomes of pregnancy such as preeclampsia, when the outcome of pregnancy was analyzed prospectively, and the rate of occurrence of preeclampsia, was compared in the

anticardiolipin antibody-positive and -negative groups, as published in obstetrics and gynecology journal (Yasuda *et al.,* 1995).

Data sources include PubMed and LILACS which were perused up to June 2009, Inclusion criteria were: cohorts, case-control, or controlled cross-sectional studies to systematically review the evidence of the association of anticardiolipin antibodies with preeclampsia. Moderate-to-high levels of anticardiolipin antibodies are associated with preeclampsia, but there is insufficient evidence to use anticardiolipin antibodies as predictors of preeclampsia in clinical practice (do prado *et al.,* 2010).

Presently, there is no obvious explanation why some individuals develop aPL, however according to the ‘second hit hypothesis, postulated by several authors, it is assumed that triggers, for example, oxidative stress, surgery, trauma or infections, which involve states of systematic inflammation and tissue damage are necessary as second hit to initiate the assemblage of immune complexes at the surface of endothelial cells.

Placental infarctions were initially thought to be the main cause of fetal loss, the direct effects of aPL on placentation and apoptosis of trophoblast cells may have more relevance, and there is mounting evidence that inflammatory state is involved in the pathophysiology of obstetric events (Antovic *et al.*, 2018).

2.6.1.Antiphspholipid syndrome and preeclampsia

Antiphospholipid antibodies are a heterogeneous population of autoantibodies against different target antigens predominately anionic phospholipid or phospholipid containing structures. Presence of antiphospholipid antibodies has been reported to have strong association with a variety of pregnancy induced complications such as preeclampsia women with high titer of antiphospholipid IgG have 28% chance of fetal loss (Melssia, Gloria, and Cynthia, 2019).

The presence of IgG antiphospholipid appears to be of greater significance than presence of IgM in detecting women at risk of fetal loss. High quality scientific data to support these associations, however lacking, and future studies are should address at least part of these uncertainties. The main limitation reported is the definition of positive aPL, with only few studies addressing this issue as recommended by international classification criteria. Standarzation of laboratory criteria and multicenter studies may help improve quality of following studies (Melssia, Gloria, and Cynthia, 2019).

APS is among the most frequent acquired risk factors to a treatable cause of recurrent pregnancy loss and increased risk of conditions associated with ischemic placental dysfunction such as preeclampsia. The most critical flaws of the studies on this association are related to methodologies applied, and definition of positively, heterogeneous definition of preeclampsia, small sample size, and lack of repeat testing. Current evidence doesn't justify inclusion of preeclampsia as a major criterion in diagnosis when a patient has other clinical features of APS (Melssia, Gloria, and Cynthia, 2019).

Chapter Three

Materials and Methods

3. Materials and Methods

3.1. Materials

3.1.1. Study design

This is a cross sectional study.

3.1.2. Study area and period

The study was conducted in Khartoum state- Sudan. The study was carried out over 4 month (Aug –Dec 2019).

3.1.3. Study population

The study was conducted on 80 Sudanese pregnant women 40 Preclamptic females as case group and 40 healthy pregnant females as controls.

3.1.4. Selection criteria

Inclusion Criteria

Pregnant women as case group with blood pressure more than 140/90 mm Hg and significant proteinuria after 20weeks and prior to 34 week of gestation with no history of renal dysfunction, and normotensive pregnant women as control group with normal renal function and no evident proteinuria upon measurement with a dipstick.

Exclusion criteria

Pregnant women with kidney disease, hematuria, and ongoing urinary tract infection, patients on diuretic therapy or with mental disorders that raise doubts regarding the subjects true willingness to participate in the study.

3.1.5. Ethical considerations

The study was approved by the scientific committee of clinical chemistry department college of Medical Laboratory Science and Technology. Then a verbal informed consent was obtained from participants.

3.1.6. Sample technique

Exactly 3ml of venous blood were collected then serum obtained by centrifugation concomitant with spot urine samples, labeled, then samples stored until use for IgG anticardiolipin antibody and urine albumin/creatinine ratio estimation respectively. The urine samples were stable for 7 days in samples containing preservative at 4C and 18C and 2 days when stored at 30C.

3.1.7. Data collection

Personal and clinical data from all participants were collected using special form of questionnaire (appendix I).

3.2. Laboratory Experiments

During specimens collection, relaxation of the tested groups was insured and after taking the samples and centrifugation, all samples were preserved and freezed until the completion of the total number and then analyzed.

3.3. Data analysis

Data was analyzed by using the SPSS computer software program version 24.The independent T. test used for comparison (P-value < 0.05) was considered significant and Pearson correlation used for correlation.

3.4. Methodology

3.4.1. Estimation of IgG anticardiolipin antibody :(appendix IV)

Principle

Indirect enzyme linked immunosorbent assay was performed, controls were made from mixed serum specimens with high, intermediate and low concentrations of IgG aCL were aliquoted and stored at -20C.

In this assay, specific antibodies (IgG aCL) in the patient sample bind to the antigen (purified cardiolipin and beta-2-glycoprotein I) coated on the surface of the reaction wells. After incubation, a washing step removed unbound and non-specifically bound serum components. Next, the enzyme conjugate (HRP-labeled anti-human IgG) was added and binds to the immobilized antibody-antigen –complexes. After incubation, a second washing step removes unbound enzyme conjugate. Then the substrate solution was added and the bound enzyme conjugate hydrolyses the substrate forming a blue colored product. Addition of an acid stops the reaction generating a yellow end-product. The intensity of the yellow color correlates with concentration of the IgG aCL and used in construction of tested sample standard curve, and unknown sample concentration was calculated using this curve, then measured photometrically at 450 nm.

3.4.2. Estimation of spot urinary albumin creatinine ratio

Immunoturbidmetric assay and kinetic alkaline picrate (Jaffe) were used to calculate the concentration of albumin and creatinine, respectively, in the urine samples, automatically on COBAS INTEGRA 400 plus analyzer. ACR was calculated as urine albumin (mg/L) / urine creatinine (mmol/L).

Principle of immunoturbidimetric assay :(appendixII)

In vitro test for the quantative immunological determination of human urine albumin. Human albumin forms a precipitate with a specific antiserum which is determined turbidimetrically at 340 nm. The COBAS INTEGRA 400 plus analyzer automatically calculates the analyte concentration. The application was described in the Tina-quant Albumin Gen.2-urine application.

Principle of Jaffe reaction method: (appendix III)

In vitro test for the quantative determination of creatinine in urine on COBAS INTEGRA systems. This Kinetic colorimetric assay is based on the Jaffe method. Urine samples are automatically prediluted 1:25 with water by the instrument, in alkaline solution creatinine forms a yellow-red complex with picrate. The rate of dye formation is proportional to the creatinine concentration in the specimen. The COBAS INTEGRA 400 plus analyzer automatically calculates the analyte concentration. The application was described in the creatinine Jaffe Gen.2-urine application.

3.5 Quality control

Pathological and normal commercially available control sera of anticardiolipin antibodies were measured to assure the accuracy of results and the precision and accuracy of method used in this study. Internal quality control was performed on COBAS INTEGRA 400 plus automated analyzer, quality assurance is based on reference control materials and calibrants, as well as repeatability and reproducibility within run and between run, the instrument performed the validity of the test automatically. (Appendix II, III)

Chapter Four
Results

Chapter Four
4- Results

In this prospection which presupposed enrollment of 80 participant, 40 preeclamptic female as case group and 40 healthy pregnant female as control to scrutinize the assessment of spot urine albumin-creatinine ratio and IgG anticardiolipin antibody among Sudanese pregnant females coinciding to criterion of the study in Khartoum state, data were analyzed statistically using computer SPSS program version 24, and the result were as follow:

Table (4-1) Mean comparison of age and blood pressure (BP) in case versus control group

Variables	Case (Mean±SD)	Control (Mean± SD)	P-value	Normal value
Age (years)	29.4±5.8	27.5±5.9	0.146	14-45 years
BP (mmHg)	124.9±7.29	102±6.7	0.000	133/92 mmHg

Table (4-2) Mean comparison of IgG anticardiolpin antibody (IgG aCL) and spot urine albumin: creatinine ratio (ACR) in case versus control group

Parameters	Case (Mean ±SD)	Control (Mean± SD)	P-value	Normal value
IgG aCL (GPL-U/ml)	0.4378 ± 0.552	0.352 ± 0.619	0.517	Less than 10 GPL-U/ml
ACR (mg/mmol)	2.42 ± 0.507	0.461 ± 0.633	0.000	<1.0 mg/mmol (<10 mg/g) is considered normal. 1-3 mg/mmol (10-30 mg/g) is considered high normal.

Independent sample T. test was used, P-value < 0.05 is considered significant.

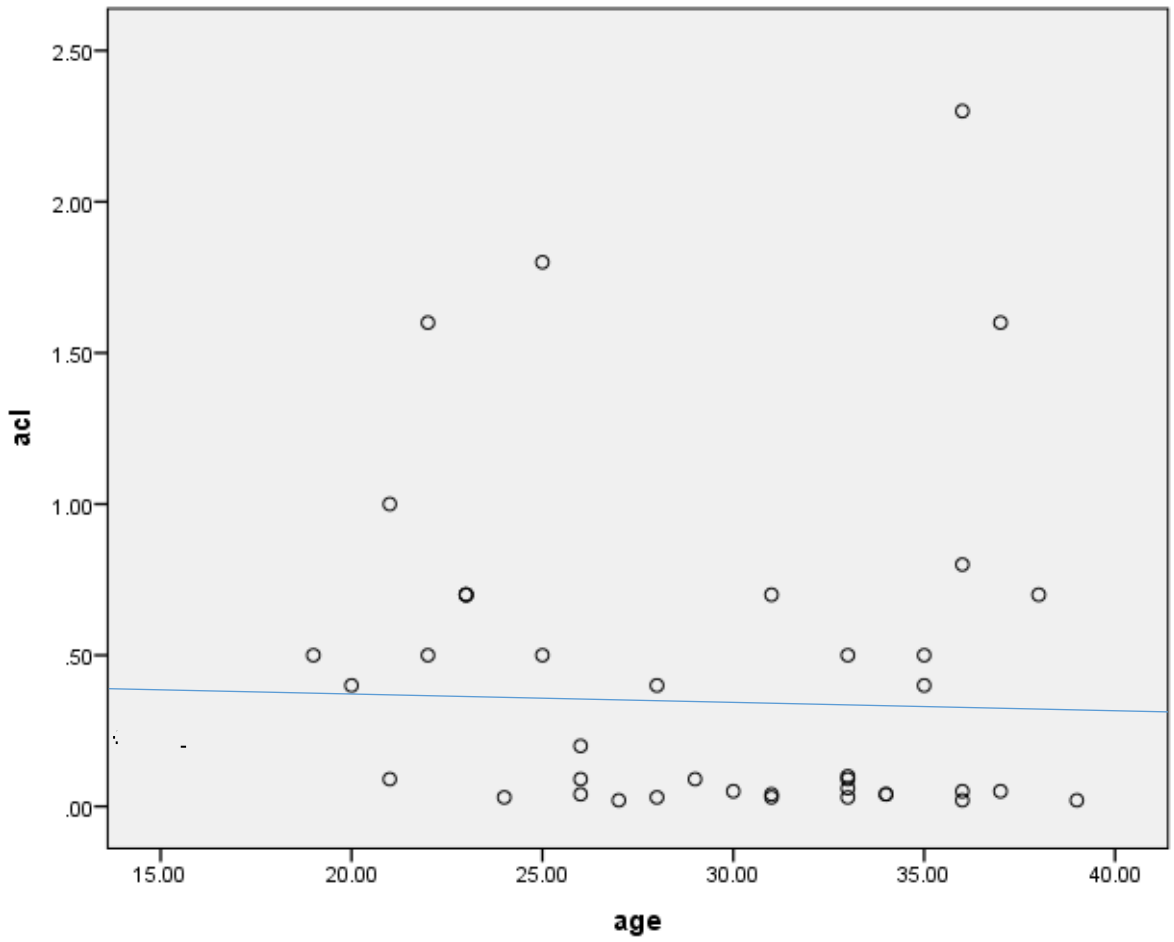

Figure (4-1): A scatter plot shows negative correlation between IgG anticardiolipin antibody level (IgG aCL) and age.

-**R**: shows the Pearson's correlation coefficient. (R= -0.091).

-**p**: shows the strings and significance of correlation. (P=0.578).

-**x** axis: Age in years.

-**Y**axis: IgG anticardiolipin antibody: GPL-U/ml.

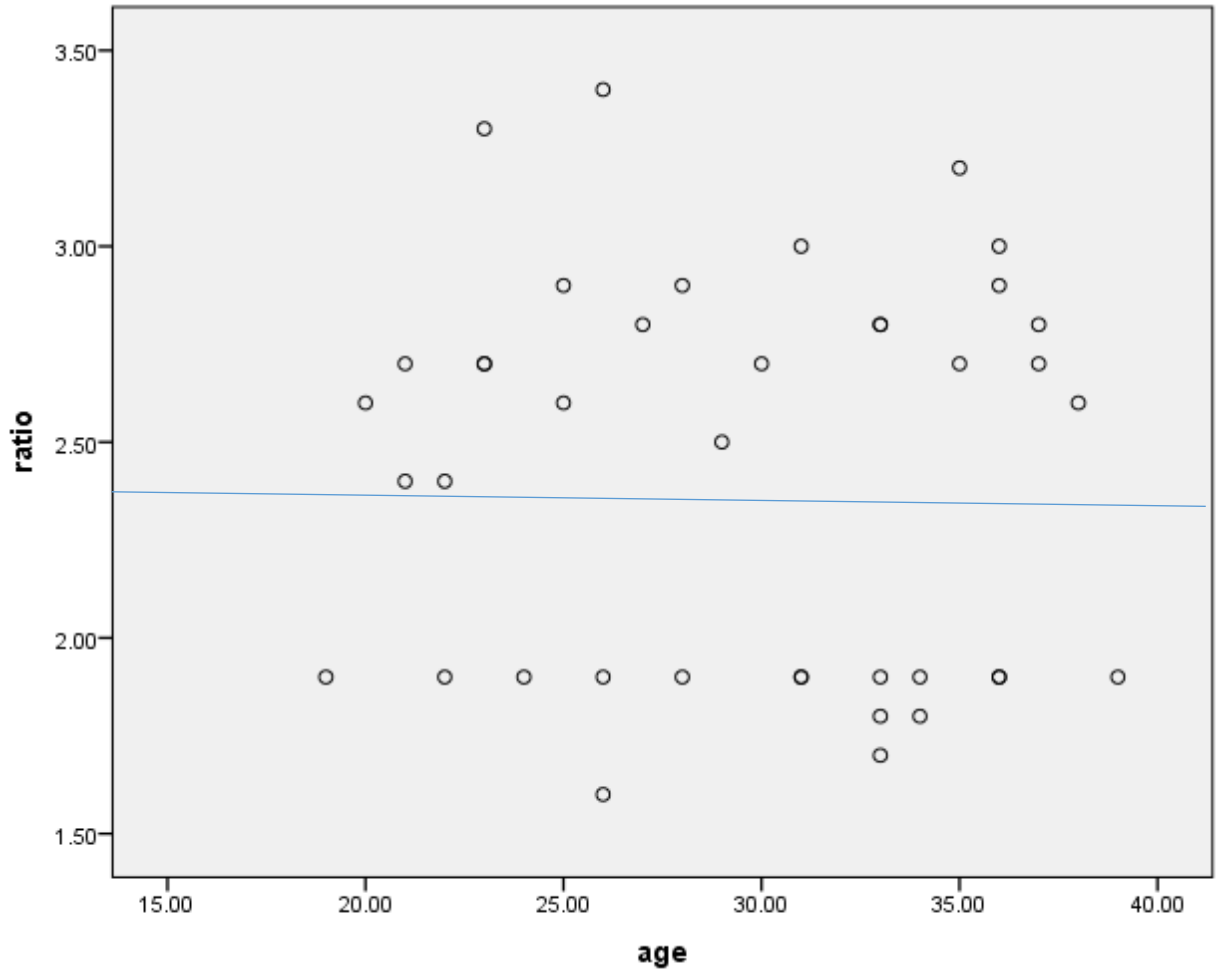

Figure (4-2): A scatter plot shows negative correlation between urine albumin: creatinine ratio (ACR) level and age.

-**R**: shows the Pearson's correlation coefficient. (R= -0.048).

-**p**: shows the strings and significance of correlation. (P=0.766).

-**x** axis: Age in years.

-**Y**axis: urine albumin: creatinine ratio: mg/mmol

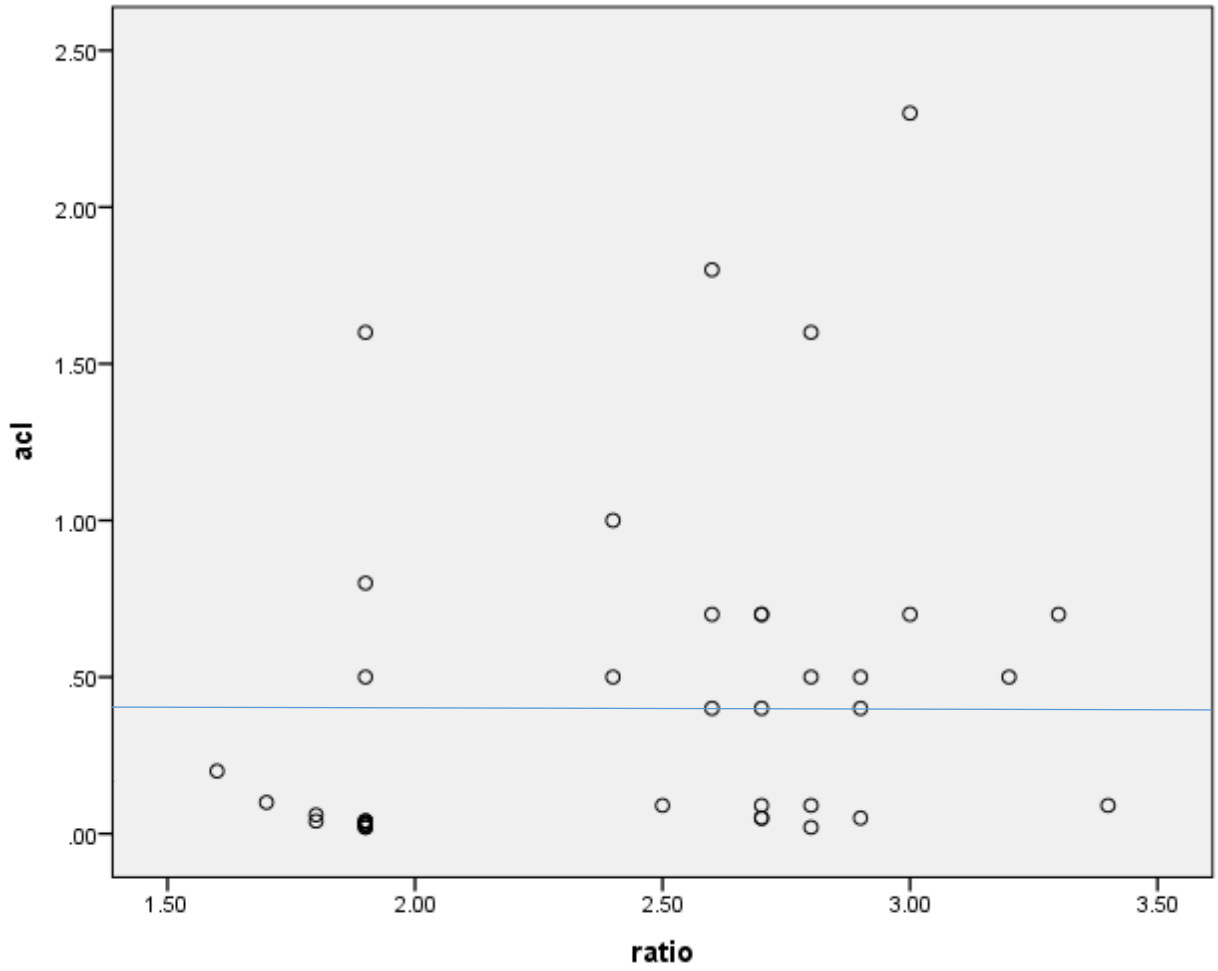

Figure (4-3): A scatter plot shows weak positive correlation between urine albumin: creatinine ratio (ACR) level and IgG anticardiolipin antibody (IgG aCL) level.

-**R**: shows the Pearson's correlation coefficient. (R=0.295).

-**p**: shows the strings and significance of correlation. (P=0.064).

-**x** axis: urine albumin: creatinine ratio: mg/mmol

-**Y**axis: IgG anticardiolipin antibody: GPL-U/ml.

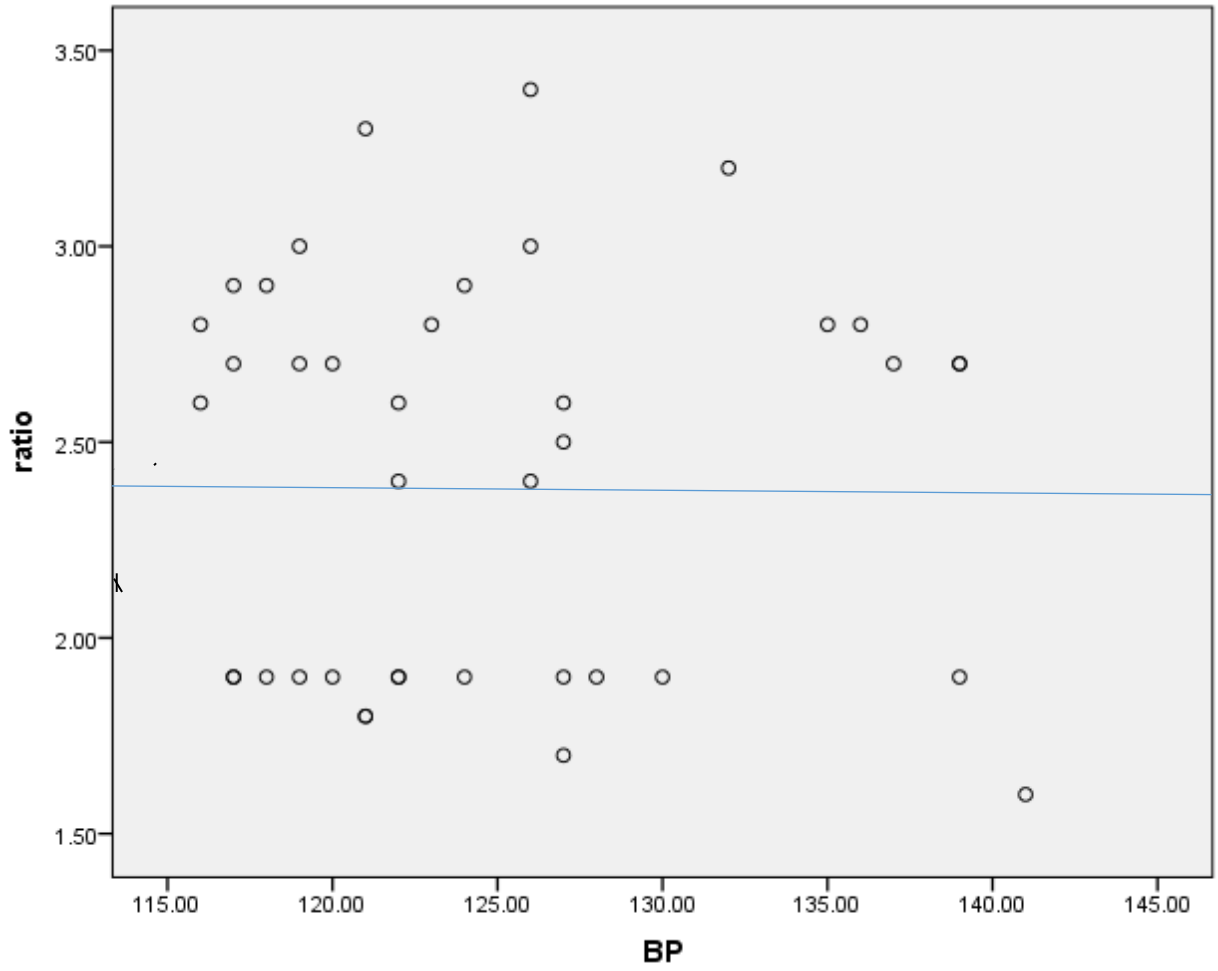

Figure (4-4): A scatter plot shows negative correlation between urine Albumin: creatinine ratio (ACR) level and blood pressure (BP).

-**R**: shows the Pearson's correlation coefficient. (R= -0.002).

-**p**: shows the strings and significance of correlation. (P=0.989).

-**x** axis: Blood pressure in mmHg.

-**Y**axis: urine albumin: creatinine ratio: mg/mmol

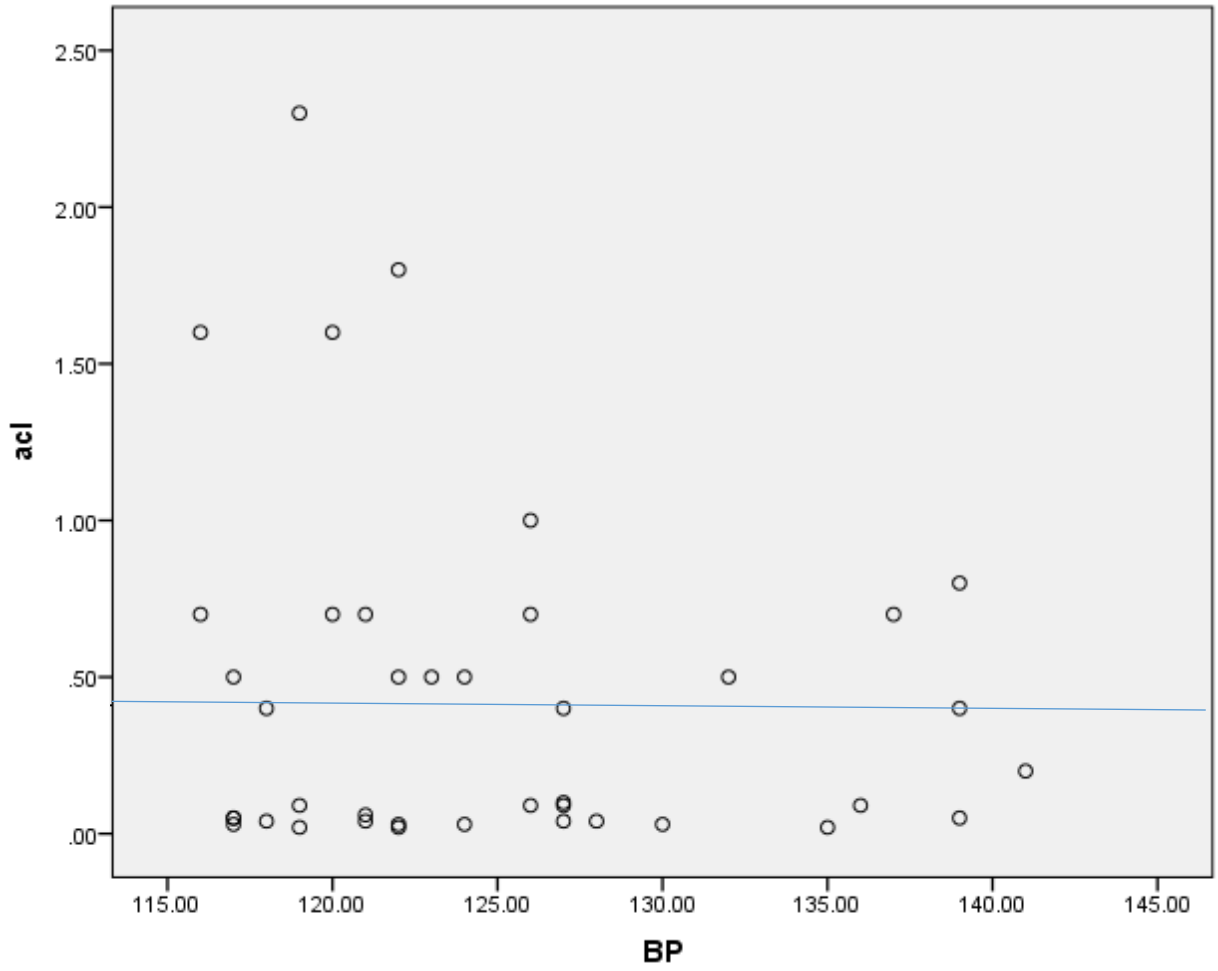

Figure (4-5): A scatter plot shows negative correlation between IgG anticardiolipin antibody (IgG aCL) level and blood pressure (BP).

-**R**: shows the Pearson's correlation coefficient. (R= -0.179).

-**p**: shows the strings and significance of correlation. (P=0.269).

-**x** axis: Blood pressure in mmHg.

-**Y**axis: IgG anticardiolipin antibody: GPL-U/ml.

Chapter Five
Discussion

5.1. Discussion

Multiple interrelated pathways have been suggested to contribute to the pathogenesis of preeclampsia which it is a multifactorial disease.

An important emerging issue is the significant linear relationship between preeclampsia and observed mortality, a phenomenon which suggests that even minimal increments of the pathological mechanisms could become clinically relevant, in the context of anticardiolipin antibody (aCL) and urine albumin: creatinine ratio (ACR), increased values has been taken to represent an early integrated markers of preeclampsia, nonetheless refutability is acceptable.

The present study revealed that the levels of anticardiolipin antibody were insignificantly decreased in preeclamptic and normotensive pregnant women with P-value (0.517), consequently the supposition of pivotal role of aCL in evolvement of preeclampsia was inadmissible. The reinforcement of our findings was clarified in a cross-sectional study published in 1999, the findings had been shown that there were no significant differences in IgG aCL between patients with preeclampsia and normotensive pregnant women, Serum aCL levels were similar in both (Abundis E *et al.,* 1999).Same conclusion observed in comparative study published in 1997(D'Anna *et al.,* 1997), nevertheless a heterogeneity among the studies which revolved on statistically significant associations between aCLs and preeclampsia had been subserved in a case-control study published in 2018 (Nielsen *et al.,* 2018).

In a cross sectional study conducted in 2007 by cardiovascular research center in Iran to assess the association between the occurrence of hypertension in pregnancy and antiphospholipid antibodies (Rawal Med, 2007), a significant high titer IgG anticardiolipin antibody was contrary to our findings which showed that there was negative correlation between anticardiolipin antibody and ambulatory blood pressure (R-value -0.179, P-value 0.269).

A significant difference in urine albumin: creatinine ratio between tested groups was verified in our study with P-value (0.000) agreed with a systematic review depend on Literature search (1980-2007) for articles of the spot albumin: creatinine ratio in hypertensive pregnancy (Anne et al, 2008), and a retrospective cohort study published in 2017 in Scotland (Elia *et al.,* 2017). Insignificant negative correlation between urine albumin: creatinine ratio and the blood pressure was observed in this study (R-value -0.002, P-value 0.989), adverse conclusion in other study aimed to look at the association between

ambulatory blood pressure and urinary albumin excretion which evaluated by determination of ACR, the mean ACR was significantly higher in hypertensive than normotensive individuals (Boulatov *et al.,* 2001).

An association was not noted in this study between the age of subjects and increment of urine albumin: creatinine ratio (R-value -0.048, P-value 0.766), also insignificant negative correlation was observed to the same variable with the occurrence of anticardiolipin antibody (R-value -0.091, P-value 0.578), and insignificant weak positive correlation was observed between ACR and aCL (R-value 0.295, P-value 0.064).

In contrast, the mean of blood pressure was significantly difference in case versus control group with P-value (0.000).

That importantly highlighted anticardiolipin antibody and urine albumin: creatinine ratio as a possible prognostic factors for preeclampsia.

5.2 Conclusion

From this study concluded that the urinary spot albumin: creatinine ratio (ACR) significantly increased in preeclamptic pregnant women, while there was no significant change concerning IgG anticardiolipin antibody (IgG aCL).

5.3 Recommendations

1. The key to prevention lies in identifying those patients most at risk of preeclampsia and closely monitoring their clinical and laboratory progress.

2. Application of prognostic models which utilize multiple prognostic factors in combination to improve individual risk prediction accuracy and discriminate between high-risk and low-risk individuals.

3. Pharmacists can play an integral role in providing early education to those expectant mothers at high risk for developing preeclampsia.

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Appendixes

Appendix I

Informed consent

الموافقة المستنيرة

انا الباحثة ستنا سليمان محمد من كلية علوم المختبرات الطبية قسم الكيمياء السريرية ، جامعة السودان للعلوم والتكنولوجيا، اقوم بدراسة بحثية لنيل درجة الماجستير بعنوان تقييم مستوى الاجسام المضادة للكارديوليبين ونسبة الالبيومين للكرياتنين في البول عند السودانيات الحوامل المصابات بما قبل تسمم الحمل بين النساء السودانيات وفقا للدراسات السابقة فان نسبة الألبيومين والكرياتينين تزيد في العينة المأخوذة من النساء اللواتي يعانين من مقدمات الارتجاع كما انه لايوجد اختلاف في مستوى الاجسام المضادة للكارديوليبين مقارنة مع الأصحاء.

.

قد تم اختيار المشاركين في البحث وفقا لإستيفائهم جميع الشروط المطلوبة للخضوع للدراسة ، فتم اختيار نساء حوامل أصحاء لايعانين من أي أمراض (ضغط دم طبيعي، غير مصابات باي إعتلال في وظائف الكلى أو الكبد)وأيضا نساء حوامل يعانين من الأعراض المراد دراسته خلال هذه الدراسة سوف نقوم بأخذ عينة بول لإجراء تحليل نسبة الالبيومين والكرياتينين و 3 مل عينة دم لإختبار الاجسام المضادة.

علما بأن سحب العينة قد يؤدي الى إحداث بعض الألم وقد يؤدي الى ظهور ورم في منطقنة الحقن يتلاشى بمرور الزمن وبحسن التعاون عند اخذ العينة يمكن تفادي كل المضاعفات.بعد الموافقة وأخذ المعلومات المتعلقة بالبحث، سوف يتم اخذ العينة والعمل على تحليلها ومن ثم إطلاعك بالنتائج المتحصل عليها خلال شهرين من زمن سحب العينة،والتي سوف تكون بسرية تامة دون الاشارة الى محددات هوية ولن يطلع عليها أحد سوى العاملين في البحث، علما بان اشتراكك سيكون طواعية مع امكانية الانسحاب في اي وقت دون خسائر، ونحيطكم علما انه لن تكون هناك اي عوائد مالية نتيجة اشتراكك وسيتم تزويدك بمعلومات وافية من النتائج التي سوف يتحصل عليها بعد استكمال الاختبارات المطلوبة التي سوف تكون مفيدة بالقدر الكافي.ويمكنك الاتصال على الباحث في الرقم.....في اي وقت للمزيد من المعلومات خلال فترة البحث.

إقرار المشاركة

بعد الإطلاع على أهداف البحث وجميع المعلومات المتعقلة به وإتاحة الفرصة كاملة لي بطرح الأسئلة وتلقي الإجابات الوافية لها ، أقر بالموافقة على المشاركة في البحث مع العلم بحقوقي بالتوقف عن المشاركة في اي وقت والاستفادة من نتائج هذه الدراسة.

توقيع المشارك: _____

ت.المشارك:.....

توقيع الباحث: _____

Sudan University of Science and Technology

College of Graduate

Studies

Questionnaire No ( )

Date: / /2019

Name……..…

Age……..…years

Weight....…Kg BMI:……..Kg/m2

BP…….mm/Hg

Term of current pregnancy……..…

History: Yes…….. No……

Pre-existing diseases…….....…….

Medications…….…….

Results:

IgG aCL…… GPL-U/ml

ACR……..…mg/mmoL

.Contact:

Phone……..…

Address……..…

ALBT2

Appendix II

cobas®

Tina-quant Albumin Gen.2 - Urine Application

Specific proteins

Order information

REF	CONTENT		Analyzers on which **cobas c** pack can be used
04469658 190	Tina-quant Albumin Gen.2 (100 tests)	System-ID 07 6743 3	COBAS INTEGRA 400 plus
03121305 122	C.f.a.s. PUC (5 × 1 mL)	System-ID 07 6755 7	
03121313 122	Precinorm PUC (4 × 3 mL)	System-ID 07 6756 5	
03121291 122	Precipath PUC (4 × 3 mL)	System-ID 07 6757 3	
20756350 322	NaCl Diluent 9 % (6 × 22 mL)	System-ID 07 5635 0	

English

System information

Test ALBU2, test ID 0-171

Intended use

In vitro test for the quantitative immunological determination of human albumin in serum, plasma, urine and cerebrospinal fluid.

The applications for serum/plasma and cerebrospinal fluid are described in the Tina-quant Albumin Gen.2 *Serum/Plasma Application* and in the Tina-quant Albumin Gen.2 *CSF Application* method sheets.

Summary[1,2,3,4,5]

Albumin is a carbohydrate-free protein, representing 55-65 % of the total plasma proteins. It maintains the plasma colloidal osmotic pressure, transports and stores a wide variety of nonpolar compounds and drugs, and serves as a source of endogenous amino acids.

The kidney normally prevents loss of serum albumin into the urine. However, albumin is still found in normal urine in small amounts. Because size (69 kD), anionic charge, and tubular reabsorption all play a role in albumin's renal handling, excretion increases with altered glomerular size and charge selectivity as well as with tubular impairment. In glomerular disease far higher amounts of albumin may be secreted than in tubular disease. Urinary albumin is therefore considered the most important marker for glomerular dysfunction. Slightly elevated albumin excretion in urine, called microalbuminuria, is of particular importance in the early diagnosis of diabetic nephropathy which develops in nearly 40 % of insulin dependent diabetes patients. The term microalbuminuria may be misleading as it seems to indicate renal excretion of a smaller albumin species, whereas it actually refers to low concentrations of normal albumin in urine. Microalbuminuria is more accurately defined as excretion above normal but lower than the detection limit of traditional dipstick tests, i.e. between 20 and 200 µg/min. Slight albuminuria and paucialbuminuria are synonyms.

Test principle[6,7]

Immunoturbidimetric assay

Human albumin forms a precipitate with a specific antiserum which is determined turbidimetrically at 340 nm.

Reagents - working solutions

R1 TRIS[a)] buffer: 50 mmol/L, pH 8.0; PEG: ≥ 4.2 %; EDTA: 2 mmol/L; preservative

R2 Polyclonal anti-human albumin antibodies (sheep): dependent on titer; TRIS[a)] buffer: 100 mmol/L, pH 7.2; preservative

SR Reagent for antigen excess check
Albumin in diluted serum (human); phosphate buffer: 50 mmol/L, pH 7.0; preservative

a) TRIS = Tris(hydroxymethyl)-aminomethane

R1 is in position A, R2 is in position B, and SR is in position C.

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods used assays approved by the FDA or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.
However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[8,9]

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C	See expiration date on **cobas c** pack label
On-board in use at 10-15 °C	12 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Urine
For qualitative albumin determination in urine use random urine sample. Use timed urine collection for quantitative analysis (24-hour urine).[10] Use only fresh urine and no preservative.

Centrifuge samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

Stability *in spontaneous, 24-hour or 2nd morning urine*[11]	7 days at 15-25 °C 1 month at 2-8 °C 6 months at (-15)-(-25) °C

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

NaCl Diluent 9 %, Cat. No. 20756350322, system-ID 07 5635 0 for automatic sample dilution and standard serial dilutions. NaCl Diluent 9 % is placed in its predefined rack position and is stable for 4 weeks on-board the COBAS INTEGRA 400 plus analyzer.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Application for urine

Test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S-R2-SR
Reaction direction	Increase
Reaction start with	R2
Wavelength A/B	340/659 nm
Calc. first/last	33/49
Typical prozone effect	> 600 mg/L (> 60 mg/dL or > 9.12 µmol/L)
Antigen excess check	Yes (with SR)
Predilution factor	No
Postconcentration factor	No
Unit	mg/L

Pipetting parameters

		Diluent (H_2O)
R1	100 µL	-
Sample	6 µL	15 µL
R2	20 µL	-
SR	6 µL	10 µL
Total volume	157 µL	

Calibration

Calibrator	C.f.a.s. PUC
Calibration dilution ratio	1:2, 1:4, 1:8, 1:16, 1:32, 1:64 performed automatically by the instrument
Calibration mode	Logit/log 4
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Enter the assigned lot-specific albumin value of the undiluted calibrator (mg/L), indicated in the package insert of C.f.a.s. PUC.

Traceability: This method has been standardized against an internal method traceable to the certified reference material in human serum of the IRMM (Institute for Reference Materials and Measurements) ERM-DA470k/IFCC.

Quality control

Quality control urine	Precinorm PUC
	Precipath PUC
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The COBAS INTEGRA 400 plus analyzer automatically calculates the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help.

Conversion factors:	mg/L x 0.1 = mg/dL
	mg/L x 0.0152 = µmol/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Icterus: No significant interference up to a conjugated bilirubin concentration of 855 µmol/L or 50 mg/dL.

Hemolysis: No significant interference up to a hemoglobin concentration of 248 µmol/L or 400 mg/dL.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[12]

No interference by acetone ≤ 60 mmol/L, ammonia chloride ≤ 0.11 mol/L, calcium ≤ 40 mmol/L, creatinine ≤ 0.18 mol/L, γ-globulin ≤ 500 mg/L, glucose ≤ 0.19 mol/L, phosphate ≤ 70 mmol/L, urea ≤ 0.8 mol/L, uric acid ≤ 5.95 mmol/L and urobilinogen ≤ 378 µmol/L.

Due to the antigen excess reagent (SR), no unflagged high-dose hook effect will occur up to an albumin concentration of 40000 mg/L (608 µmol/L).

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

3.0-200 mg/L (0.05-3.10 µmol/L or 0.3-20.0 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation:

Limit of Blank	= 2 mg/L (0.030 µmol/L or 0.200 mg/dL)
Limit of Detection	= 3 mg/L (0.046 µmol/L or 0.300 mg/dL)
Limit of Quantitation	= 12 mg/L (0.182 µmol/L or 1.20 mg/dL)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a total error of 30 %. It has been determined using low concentration albumin samples.

Values below the Limit of Quantitation (< 12 mg/L) will not be flagged by the instrument.

Expected values

2nd morning urine:[13]

Adults:	< 20 mg albumin/g creatinine or < 2.26 g (34.35 µmol) albumin/mol creatinine
Children (3-5 years):[14]	< 20 mg/L (0.304 µmol/L) albumin or < 30 mg albumin/g creatinine
24-hour urine:[15]	< 20 mg/L (0.304 µmol/L) < 30 mg/24 h (0.456 µmol/24 h)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (1 aliquot per run, 1 run per day, 21 days). The following results were obtained:

Repeatability	*Mean mg/L (µmol/L)*	*SD mg/L (µmol/L)*	*CV %*
Urine low	22.0 (0.334)	0.3 (0.004)	1.2
Urine high	115 (1.75)	2 (0.03)	1.9
Control (normal)	23.4 (0.356)	0.2 (0.003)	0.9
Control (pathological)	88.8 (1.35)	1.0 (0.02)	1.1

Intermediate precision	*Mean mg/L (µmol/L)*	*SD mg/L (µmol/L)*	*CV %*
Urine low	22.5 (0.342)	0.4 (0.006)	1.9
Urine high	118 (1.79)	3 (0.04)	2.2
Control (normal)	23.6 (0.359)	0.3 (0.011)	1.1
Control (pathological)	90.3 (1.37)	1.1 (0.02)	1.2

Method comparison

Albumin values for human urine samples obtained on a COBAS INTEGRA 800 analyzer using the COBAS INTEGRA Tina-quant Albumin Gen.2 reagent (y) were compared with those determined using the same reagent on a **cobas c** 501 analyzer (x).

cobas c 501 analyzer	Sample size (n) = 75
Passing/Bablok[16]	Linear regression
y = 1.057x - 5.48 mg/L	y = 1.047x - 5.35 mg/L
τ = 0.951	r = 0.994

The sample concentrations were between 6.50 and 181 mg/L (0.650 and 18.1 mg/dL or 0.099 and 2.75 µmol/L).

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

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CONTENT	Contents of kit
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CE

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

Roche

00048107161 90COINV10.0

CREJ2

Creatinine Jaffé Gen.2 - Urine

cobas®

Substrates

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04810716 190	Creatinine Jaffé Gen.2 (700 tests)	System-ID 07 6928 2	COBAS INTEGRA 400 plus
10759350 190	Calibrator f.a.s. (12 x 3 mL)	System-ID 07 3718 6	
10759350 360	Calibrator f.a.s. (12 x 3 mL, for USA)	System-ID 07 3718 6	
03121313 122	Precinorm PUC (4 x 3 mL)	System-ID 07 6756 5	
03121291 122	Precipath PUC (4 x 3 mL)	System-ID 07 6757 3	

English

System information

Test CRJ2U, test ID 0-546

Intended use

In vitro test for the quantitative determination of creatinine in urine on COBAS INTEGRA systems.

Summary[1,2,3,4,5]

Chronic kidney disease is a worldwide problem that carries a substantial risk for cardiovascular morbidity and death. Current guidelines define chronic kidney disease as kidney damage or glomerular filtration rate (GFR) less than 60 mL/min per 1.73 m^2 for three months or more, regardless of cause.

The assay of creatinine in serum or plasma is the most commonly used test to assess renal function. Creatinine is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass). It is freely filtered by the glomeruli and, under normal conditions, is not re-absorbed by the tubules to any appreciable extent. A small but significant amount is also actively secreted.

Since a rise in blood creatinine is observed only with marked damage of the nephrons, it is not suited to detect early stage kidney disease. A considerably more sensitive test and better estimation of glomerular filtration rate (GFR) is given by the creatinine clearance test based on creatinine's concentration in urine and serum or plasma, and urine flow rate. For this test a precisely timed urine collection (usually 24 hours) and a blood sample are needed. However, since this test is prone to error due to the inconvenient collection of timed urine, mathematical attempts to estimate GFR based only on the creatinine concentration in serum or plasma have been made. Among the various approaches suggested, two have found wide recognition: that of Cockroft and Gault and that based on the results of the MDRD trial. While the first equation was derived from data obtained with the conventional Jaffé method, a newer version of the second is usable for IDMS-traceable creatinine methods. Both are applicable for adults. In children, the Bedside Schwartz formula should be used.[6,7,8,9]

In addition to the diagnosis and treatment of renal disease, the monitoring of renal dialysis, creatinine measurements are used for the calculation of the fractional excretion of other urine analytes (e. g., albumin, α-amylase). Numerous methods were described for determining creatinine. Automated assays established in the routine laboratory include the Jaffé alkaline picrate method in various modifications, as well as enzymatic tests.

Test principle[10,11,12]

This kinetic colorimetric assay is based on the Jaffé method. In alkaline solution, creatinine forms a yellow-red complex with picrate. The rate of dye formation is proportional to the creatinine concentration in the specimen.

Creatinine + picric acid $\xrightarrow{\text{Alkaline pH}}$ yellow-red complex

Reagents - working solutions

R1 Potassium hydroxide: 900 mmol/L; phosphate: 135 mmol/L; pH ≥ 13.5

SR Picric acid: 38 mmol/L; pH 6.5; non reactive buffer

R1 is in position B and SR is in position C.

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Danger

H314	Causes severe skin burns and eye damage.
EUH 001	Explosive when dry

Prevention:

P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.

Response:

P301 + P330 + P331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.
P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor.
P305 + P351 + P338 + P310	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590, USA: 1-800-428-2336

Reagent handling

Ready for use

Storage and stability

Shelf life at 15-25 °C	See expiration date on **cobas c** pack label
On-board in use at 10-15 °C	8 weeks

Specimen collection and preparation[13]

For specimen collection and preparation only use suitable tubes or collection containers.

Collect urine without using additives. If urine must be collected with a preservative for other analytes, only hydrochloric acid (14 to 47 mmol/L urine, e.g. 5 mL 10 % HCl or 5 mL 30 % HCl per liter urine) or boric acid (81 mmol/L, e.g. 5 g per liter urine) may be used.

Urine samples are automatically prediluted 1:25 (1 + 24) with water by the instrument.

See the limitations and interferences section for details about possible sample interferences.

Stability without preservative:[14]	2 days at 15-25 °C
	6 days at 2-8 °C
	6 months at (-15)-(-25) °C
Stability with preservatives:	3 days at 15-25 °C
	8 days at 2-8 °C
	3 weeks at (-15)-(-25) °C

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Materials provided

See "Reagents – working solutions" section for reagents.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Application for urine

Test definition

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction direction	Increase
Wavelength A/B	512/583 nm
Calc. first/last	40/49
Reaction mode	D-R1-S-SR
Predilution factor	25
Unit	mmol/L

Pipetting parameters

		Diluent (H_2O)
R1	13 µL	71 µL
Sample	10 µL	20 µL
SR	17 µL	16 µL
Total volume	147 µL	

Calibration

Calibrator	Calibrator f.a.s.
	Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each **cobas c** pack, every 7 days, and as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against ID/MS.[a)]

For the USA, this method has been standardized against a primary reference material (SRM[b)] 914 and SRM 967 (ID/MS)).

a) Isotope Dilution Mass Spectrometry

b) Standard Reference Material

Quality control

Reference range	Precinorm PUC
Pathological range	Precipath PUC
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The COBAS INTEGRA 400 plus analyzer automatically calculates the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help.

Conversion factor: mmol/L x 11.3 = mg/dL

Limitations - interference

Criterion: Recovery in the creatinine decision range for adults (20 mmol/L in urine) within ± 10 % of initial value.

Icterus: No significant interference up to a bilirubin concentration of 855 µmol/L or 50 mg/dL.

Hemolysis: No significant interference up to a hemoglobin concentration of 683 µmol/L or 1100 mg/dL.

Glucose: No significant interference from glucose up to a concentration of 117 mmol/L (2100 mg/dL).

Urobilinogen: No significant interference from urobilinogen up to a concentration of 676 µmol/L (40 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[15] Exception: Hydroxocobalamin (Cyanokit) may cause artificially low results.

Criterion: Recovery within ± 10 % of initial value at a creatinine concentration of 2500 µmol/L (28.3 mg/dL).

Urea: No significant interference from urea up to a concentration of 2100 mmol/L (12612 mg/dL).

High homogentisic acid concentrations in urine samples lead to false results.

The presence of ketone bodies can cause artificially high results in urine.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[16]

Estimation of the Glomerular Filtration Rate (GFR) on the basis of the Schwartz Formula can lead to an overestimation.[17]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

0.027-32.5 mmol/L (0.31-367 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:4 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 4.

Lower limits of measurement

Lower detection limit of the test:
0.027 mmol/L (0.31 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of a zero sample (zero sample + 3 SD, repeatability, n = 30).

Expected values

1st morning urine[18]		
Females	2.47-19.2 mmol/L	(28-217 mg/dL)
Males	3.45-22.9 mmol/L	(39-259 mg/dL)
24 h urine[19]		
Females	7-14 mmol/24 h	(740-1570 mg/24 h)
Males	9-21 mmol/24 h	(1040-2350 mg/24 h)
Creatinine clearance[19,20]		71-151 mL/min

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability and intermediate precision (2 aliquots per run, 2 runs per day, 20 days). The following results were obtained:

	Level 1	Level 2
Mean	2.16 mmol/L (24.4 mg/dL)	19.1 mmol/L (216 mg/dL)
CV repeatability	1.4 %	0.8 %
CV intermediate precision	2.5 %	1.6 %

Method comparison

Creatinine values for human urine samples obtained on a COBAS INTEGRA 700 analyzer with the COBAS INTEGRA Creatinine Jaffé Gen.2 reagent (y) were compared with those determined using the commercially available reagent for creatinine on an alternative manufacturer's clinical chemistry system (x). Samples were measured in duplicate. Sample size (n) represents all replicates.
Sample size (n) = 150

Alternative system	
Passing/Bablok[21]	Linear regression
y = 1.04x - 0.01 mmol/L	y = 1.04x + 0.02 mmol/L
τ = 0.963	r = 0.999
SD (md 95) = 0.388	Sy.x = 0.241

The sample concentrations were between 2.0 and 21.9 mmol/L (22.6 and 247 mg/dL).

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Symbols

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CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

Additions, deletions or changes are indicated by a change bar in the margin.

CREJ2

Creatinine Jaffé Gen.2 - Urine

cobas®

Substrates

CE

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support 1-800-428-2336

Roche

Appendix IV

ORGENTEC Diagnostika GmbH
Carl-Zeiss-Straße 49-51
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Fax: +49 (0) 61 31 / 92 58-58
Internet: www.orgentec.com

ORGENTEC

Instruction For Use
2013-12

CE

ORG 515 Anti-Cardiolipin IgG/IgM

NAME AND INTENDED USE

Anti-Cardiolipin IgG/IgM is an ELISA test system for the quantitative measurement of IgG and IgM class autoantibodies against cardiolipin in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
96	Sufficient for 96 determinations
LOT	Batch code
	Use by
	Temperature limitation
	Consult instructions for use
	Keep away from sunlight
	Do not reuse
	Date of manufacture

MICROPLATE	Microplate
CALIBRATOR A	Calibrator
CALIBRATOR B	Calibrator
CALIBRATOR C	Calibrator
CALIBRATOR D	Calibrator
CALIBRATOR E	Calibrator
CALIBRATOR F	Calibrator
CONTROL +	Control positive
CONTROL −	Control negative
DILUENT	Sample Buffer P
CONJUGATE G	Enzyme Conjugate
CONJUGATE M	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use

PRINCIPLE OF THE TEST

Highly purified cardiolipin is coated on microwells saturated with beta-2-glycoprotein I.
The determination is based on an indirect enzyme linked immune reaction with the following steps:
Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subesquently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyses the substrate forming a blue coloured product. Addition of an acid stopps the reaction generating a yellow end-product. The intensity of the yellow color
correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

CONTENTS OF THE KIT

ORG 515	▽ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: CLP
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 GPL-U/ml / 0 MPL-U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 7.5 GPL-U/ml / 5 MPL-U/ml, containing Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent,NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 15 GPL-U/ml / 10 MPL-U/ml, containing Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 30 GPL-U/ml / 20 MPL-U/ml, containing Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 60 GPL-U/ml / 40 MPL-U/ml, containing Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 120 GPL-U/ml / 80 MPL-U/ml, containing Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
CONTROL −	1x 1.5 ml	Control negative, containing cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate 5x.
CONJUGATE G	15 ml	Enzyme Conjugate IgG, containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
CONJUGATE M	15 ml	Enzyme Conjugate IgM; containing anti-human IgM antibodies, HRP labelled, PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate, containing 3,3', 5,5'- Tetramethylbenzidin. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%, 50 x conc.
	1	Instruction for Use: ELISA Mini-DVD
	1	Certificate of Analysis

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm, optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

deionised water to a final volume of 100 ml.

Preparation of samples

Dilute patient samples 1:100 before the assay. Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators / Controls are ready to use and need not be diluted.

TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette **100 µl of** calibrators, controls and prediluted patient samples into the wells.
   Incubate for **30 minutes** at room temperature (20-28 °C).
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
2. Dispense **100 µl** of enzyme conjugate into each well.
   Incubate for **15 minutes** at room temperature.
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
3. Dispense **100 µl** of TMB substrate solution into each well.
   Incubate for **15 minutes** at room temperature.
4. **Add 100 µl** of stop solution to each well of the modules.
   Incubate for **5 minutes** at room temperature.
   Read the optical density at 450 nm (reference 600-690nm) and calculate the results.
   The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P1	A	P1								
B	B	P2	B	P2								
C	C	P3	C	P3								
D	D	P4	D	P4								
E	E	P5	E	P5								
F	F	P6	F	P6								
G	C+	P7	C+	P7								
H	C-	P8	C-	P8								
	IgG	IgG	IgM	IgM								

P1, ... patient sample A-F calibrators C+, C- controls

VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit.
If these quality control criteria are not met the assay run is invalid and should be repeated.

CALCULATION OF RESULTS

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation.
Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

PERFORMANCE CHARACTERISTICS

Calibration

The assay system is calibrated against the internationally recognised reference sera from E.N. Harris, Louisville and the specific reference material IRP 97/656 (IgG) and HCAL (IgG) / EY2C9 (IgM).

Measuring range

The calculation range of this ELISA assay is: IgG: 0 - 120 GPL-U/ml IgM: 0 - 80 MPL-U/ml

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off IgG: 10 GPL-U/ml IgM: 7 MPL-U/ml

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1. Pipet **100 µl** calibrator, control or patient sample
   → Incubate for **30 minutes** at room temperature
   → Discard the contents of the wells and wash 3 times with **300 µl** wash solution
2. Pipet **100 µl** enzyme conjugate
   → Incubate for **15 minutes** at room temperature
   → Discard the contents of the wells and wash 3 times with **300 µl** wash solution
3. Pipet **100 µl** substrate solution
   → Incubate for **15 minutes** at room temperature
4. Add **100 µl** stop solution
   → Leave untouched for **5 minutes**
   → Read at **450 nm**