Sudan University of Science and Technology

College of Graduate Studies

The Utility of Serum Total and Placental Alkaline Phosphatase Activity (ALP) as a Predictive Marker for Preeclampsia among Sudanese Pregnant Women in Khartoum State.

فائدة إنزيم الفوسفاتير القلوي الكلي والمشيمي في الدم كمؤشر لتسمم الحمل لدى الحوامل السودانيات في ولاية الخرطوم.

A dissertation submitted in partial fulfillment for the requirement of MSc Degree in Medical Laboratory Sciences Clinical Chemistry

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الآيـــــة

قال تعالى:

﴿ لَقَدْ خَلَقْنَا الْإِنْسَانَ فِي كَبَدٍ﴾

[سورة البلد الآية 4]

Dedication

To my beloved mother….(Mathani Al amin)

Who encouraged me at all stages of my life.

To my sisters…..(Enas, Lujain)

for there unlimited support.

Aacknowledgements

supervisor I wish to express my sincere gratitude and thankfulness to my **Dr.Ghada Abdelrhman Elfadil** for her patience, guidance, meticulous supervision, revision , suggestion and discussing all aspects of this study . Hervaluable advices and comments are highly appreciated .

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Abstract

Background and Aim: Preeclampsia is a potentially serious complication of pregnancy with increasing significance worldwide. About 2-fold increased ALP in Preeclampsia groups indicative of placental dysfunction, Serum total alkaline phosphatase (ALP) and placenta alkaline phosphatase (PALP) measurement can afford a simple, reliable and economical adjunctive laboratory parameter in hypertensive disorders of pregnancy. The aim of this study was to determine the utility of serum and placental ALP as a Predictive marker for preeclampsia among Sudanese pregnant females.

Materials and Methods: This cross-sectional comparative hospital-based study conducted in Khartoum state at Omdurman Maternity Hospital form March to June 2022. Hundred Sudanese pregnant women aged between (15-45) years old were included, of them 50 pregnant women with preeclampsia and 50 apparently healthed pregnant women as control. The serum TALP and PALP was measured by using full automation analyser (response® 910). The data obtained was analyzed by using SPSS version 20 program.

Results: The study showed insignificant difference in mean of total ALP in preeclamptic group compared to normal pregnant women (P.value > 0.05), However there was significant increase in means of PALP (Mean±SD 151.5± 76.1IU/L vs 111.1±65.4 IU/L, P.value < 0.05) and the ratio of PALP/TALP (mean ±SD 0.66 ±0.2 IU/L vs 0.52±0.1 IU/L, P.value < 0.05) in preeclamptic group when compared to normal pregnant women. The Receiver operating characteristics curve (ROC) showed an optimum cut offs PALP (133 IU/L) and ratio (0.589 IU/L) in predictor of preeclampsia in pregnancy.

Conclusion: Sudanese women with preeclampsia had elevated serum PALP and ratio of PALP/TALP. Moreover PALP and ratio of PALP/TALP can be used as predictive marker of preeclampsia in pregnancy.

المستخلص

الخلفية والهدف: تسمم الحمل من المضاعفات الخطيرة المحتملة للحمل مع زيادة الأهمية في جميع أنحاء العالم. حوالي 2 أضعاف زيادة ALP في مجموعات تسمم الحمل يدل على خلل في المشيمة ، يمكن أن يوفر قياس الفوسفاتيز القلوي الكلي TALP والفوسفاتيز القلوي المشيمي PALP في المصل معلومات مختبرية بسيطة وموثوقة واقتصادية في اضطرابات ارتفاع ضغط الدم أثناء الحمل. الهدف من هذه الدراسة هو تحديد الفائدة من ALP في المصل والمشيمة كعلامة تنبؤيه لتسمم الحمل لدى النساء السودانيات الحوامل.

المواد والطرق: هذه دراسة مقارنة مقطعية لقاعدة مستشفيات أجريت في ولاية الخرطوم بمستشفى أم درمان للولادة من مارس إلى يونيو 2022. مئه امرأة حامل سوداني تتراوح أعمارهن بين (15-45) عامًا ، منهن 50 امرأة حامل مصابة بتسمم الحمل و 50 امرأة حامل صحيًا كعنصر تحكم. تم قياس TALPوPALP في الدم باستخدام التحليل الآلي الكامل (response® 910). تم تحليل البيانات التي تم الحصول عليها باستخدام برنامج SPSS الإصدار 20.

النتائج: اوضحت الدراسه عدم الإختلاف في متوسط ALPالكلي في النساء المصابات بتسمم الحمل مقارنة بالنساء الطبيعيات الحوامل(يعني SD± 221.8 ± 76.4 وحدة دولية / لتر مع 204.06 ± 93.2 وحدة دولية / لتر > P.value 0.05) . ومع ذلك كانت هناك زياده واضحه في متوسطات PALP(يعني ± SD 151.5 ± 76.1 وحدة دولية / لتر مع 111.1 ± 65.4 وحدة دولية / لتر) و نسبة PALP/TALP (يعني ± SD 0.66 ± 0.2IU/L ،0.52± 0.1 IU/L ,0.05>P.value) في النساء المصابات بتسمم الحمل مقارنة بالنساء الطبيعيات الحوامل. أظهر منحنى خصائص تشغيل جهاز الاستقبال (ROC) لـ PALP قطعًا مثاليًا عند(133 وحده دولية/ لتر) ونسبه PALP/TALP (0.589 وحده دولية/لتر) كعلامه تنبؤيه لتسمم الحمل أثناء الحمل.

الخلاصة: النساء السودانيات المصابات بتسمم الحمل لديهن ارتفاع في PALP و نسبة PALP/TALP في الدم . لذلك يمكن استخدام PALP و نسبة PALP/TALP كعلامة تنبؤيه لتسمم الحمل اثناء الحمل.

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List of Abbreviations:

AUC: Area Under the Curve.

DBP: Diastolic Blood Pressure.

EC: Enzyme Commission number.

HDP: Hypertension Disorder of Pregnancy.

IU/L: International unit/ Liter.

PALP: Placenta Alkaline Phosphatase.

PE: Preeclampsia.

ROC: Receiver operating Characteristic Curve.

SBP: Systolic Blood Pressure.

SD: Stander Deviation.

SPSS: statistical package of social science.

TALP: Total Alkaline Phosphatase.

Chapter One

Introduction, Rationale, Objectives

1. Introduction, Rationale and Objectives

1.1 Introduction

Preeclampsia is a potentially serious complication of pregnancy with increasing significance worldwide. Preeclampsia is the cause of 9%–26% of global maternal mortality and a significant proportion of preterm delivery, and maternal and neonatal morbidity (***Townsend et al.,2016***).

Hypertension disorder in pregnancy (HDP) is defined as a systolic blood pressure ≥ 140 or diastolic blood pressure ≥ 90 mmHg or both . HDP comprise a spectrum of disorders Categorized into Gestational hypertension defined as new hypertension presenting at or after 20 weeks gestation without proteinuria or other features of preeclampsia, preeclampsia is hypertension with significant proteinuria, when preeclampsia develops in women with chronic hypertension, the classification is chronic hypertension with superimposed preeclampsia and eclampsia, chronic (pre-existing) hypertension is hypertension that is present before 20 weeks of gestation or prior to pregnancy (***Reeta et al.,2014. Chaparro et al.,2020*).**

Incidence is increasing in keeping with the increase in obesity, maternal age, and women with medical comorbidities entering pregnancy. Recent developments in the understanding of the pathophysiology of preeclampsia have opened new avenues for prevention, screening, and management of this condition. In addition, it is known that preeclampsia is a risk factor for cardiovascular disease in both the mother and the child and presents an opportunity for early preventative measures. New tools for early detection, prevention, and management of preeclampsia have the potential to revolutionize practice in the coming years (***Townsend et al.,2016***).

Preeclampsia is a global health problem of increasing significance. Preeclampsia complicates 2%–8% of all pregnancies, contributes to 15% of preterm deliveries. In the most recent confidential inquiry into maternal mortality in the UK, 22 of 107 direct maternal deaths from 2006 to 2008 were related to preeclampsia and eclampsia. (***Townsend et al.,2016***).

Several biochemical parameters, serum albumin, total protein, iron, iron binding capacity, hemoglobin, ferritin, uric acid, Magnesium, aminotransferases, total Alkaline phosphatase (ALP), Placenta Alkaline phosphatase (PALP) and urinary protein had been cited in the past which find wide use in predicting pregnancy hypertension. In recent years, however certain novel biochemical substances such as prostaglandins, Inhibin, Cell Adhesion Molecules have been shown to possess clinical relevance in monitoring the course and outcome of hypertensive disorders of pregnancy (***Reeta et al.,2014***).

Heat stable ALP (PLAP) measurement can afford a simple, reliable and economical adjunctive laboratory parameter in hypertensive disorders of pregnancy (***Reeta et al.,2014***).

1.2 Rationale

and Preeclampsia is becoming an increasingly common diagnosis in the developed world remains a high cause of maternal and fetal morbidity and mortality in the developing world and known that preeclampsia is a risk factor for cardiovascular disease in both the mother and the child and presents an opportunity for early preventative measures. New tools for early detection, prevention, and management of preeclampsia have the potential to revolutionize practice in the coming years**,** till date, studies on placental alkaline phosphatase (heat stable fraction) as a marker in diagnosis of preeclampsia is sparse, so this study conducted to determine the diagnostic value of ALP and PALP as a reliable, sensitive, specific and economical biochemical marker for preeclampsia.

1.3 Objectives

1.3.1 General Objective

To determine the utility of serum total and placental ALP as a Predictive marker for preeclampsia among Sudanese pregnant women in Khartoum state

1.3.2 Specific Objectives

1.To estimate and compare the level of analytes under study (Total ALP and PALP) in preeclamptic pregnant women and normal apparent pregnant women .

2.To correlate between study parameters (total ALP, PALP and ratio of PALP/TALP) and study variables (Age, SBP, DBP, gravida).

3.To determine the sensitivity and specificity of total ALP, PALP and ratio of PALP/TALP as predictor of preeclampsia in Sudanese pregnant women.

Chapter Two

Literature Review

2.Literature Review

2.1 Preeclampsia

Pre-eclampsia (PE) is defined as the new onset of hypertension and proteinuria or as the new onset of hypertension and significant end-organ dysfunction with or without proteinuria after 20 weeks of gestation in a previously normotensive woman. This hypertensive disorder complicates 3–5% of all pregnancies and is one of the leading causes of maternal morbidity and mortality. The severity of adverse outcomes is strongly associated with gestational age at onset. In approximately 90% of cases, PE onset after 34 weeks of gestation is associated with good health outcomes, although the mother and newborn are at increased risk of serious morbidity or mortality when compared to normotensive pregnancies. Early presentation of PE (i.e., <34 weeks) is associated with poor placentation and dysfunctional spiral artery remodeling and greater risk of adverse outcome, and it is associated with moderately preterm, very preterm, or extremely preterm birth. In addition, long-term, women who developed PE are at increased risk of developing cardiovascular and renal diseases ***(Chaparro et al.,2020)***.

Preeclampsia has been dubbed a disease of theories. Its concept has transformed throughout the century from a disease specific to the kidney leading to chronic nephritis to a state of toxemia caused by circulating toxins. Our understanding of this disorder has significantly advanced since that time: the introduction of circulating antiangiogenic factors contributing to disease and the emphasis away from proteinuria to diagnose preeclampsia. What have also been unraveled, more so in the last decade, are the future cardiovascular and renal implications for women with a history of preeclampsia, especially those with early, severe subtypes. Thus, with much better understanding of this disease, we have optimism for diagnosis and treatment as well as caution for future care of these women (***Elizabeth et al.,2016)***.

The early identification of women at risk of PE would allow for the development and evaluation of timely intervention strategies to limit immediate and long-term adverse outcomes. Multiparametric algorithms for the identification of women at risk of developing PE have been previously reported; they are based on combinations of maternal risk factors, uterine artery Doppler pulsatility, and/or different blood-borne biomarkers (***Chaparro et al.,2020***).

2.1.1 Epidemiology of Preeclampsia

Hypertensive disorders of pregnancy, including preeclampsia, consist of a broad spectrum of conditions that are associated with substantial maternal and fetal/neonatal morbidity and mortality. The incidence of hypertensive disorders in pregnancy is estimated to range between 3% and 10% among all pregnancies. Worldwide, preeclampsia and related conditions are among the leading causes of maternal mortality. While maternal death due to preeclampsia is less common in developed countries, preeclampsia-related maternal morbidity is high and remains a major contributor to intensive care unit admissions during pregnancy. Studies have reported a 7–20% chance of preeclampsia recurrence in a subsequent pregnancy. This risk is further increased if a woman has had two prior preeclamptic pregnancies and is also influenced by the gestational age of onset. One-quarter of stillbirths and neonatal deaths in developing countries are associated with preeclampsia/eclampsia. Approximately 12–25% of growth-restricted fetuses and small for gestational age infants as well as 15–20% of all preterm births are attributable to preeclampsia; the associated complications of prematurity are substantial and include neonatal deaths and serious long-term neonatal morbidity. One-quarter of stillbirths and neonatal deaths in developing countries are associated with preeclampsia/eclampsia. Infant mortality associated with preeclampsia is three times higher in low-resource settings than in high-income countries, largely due to the lack of neonatal intensive care facilities. Despite major medical advances, the only known cure for preeclampsia remains delivery of the fetus and placenta (***Jeyabalan A,2013***). A systematic review by the World Health Organization indicates that hypertensive disorders account for

16% of all maternal deaths in developed countries, 9% of maternal deaths in Africa and Asia, and as many as 26% of maternal deaths in Latin America and the Caribbean. Where maternal mortality is high, most of the deaths are attributable to eclampsia rather than preeclampsia. Based on data from the United States National Hospital Discharge Survey, the prevalence of preeclampsia during admission for labor and delivery increased by 25% from 1987 to 2004; during the same period, the rate of eclampsia decreased by 22%, but this was not statistically significant. Severe morbidity associated with preeclampsia and eclampsia includes renal failure, stroke, cardiac dysfunction or arrest, respiratory compromise, coagulopathy, and liver failure. In a study of hospitals managed by Health Care America Corporation, preeclampsia was the second leading cause of pregnancy-related intensive care unit admissions, after obstetric hemorrhage (***Jeyabalan A,2013***).

2.1.2 Pathophysiology of Preeclampsia

Multiple etiologies have been proposed to play a role in the pathophysiology of PE, principally related to an abnormal placentation and utero placental ischemia, that in turn are associated with an increased release of cellular debris from the trophoblast into the maternal circulation that contributes to systemic inflammation, endothelial dysfunction, and the clinical manifestation of the disease (***Alejandra et al.,2021***). Until now, the only effective treatment of PE is preterm delivery of the fetus, thus removing the deleterious effects of the placenta on maternal physiology (***Brown D,2020. Alejandra et al.,2021***).

The pathophysiologic processes underlying this disorder are described as occurring in two stages. The first stage is characterized by reduced placental perfusion, possibly related to abnormal placentation, with impaired trophoblast invasion and inadequate remodeling of the uterine spiral arteries. The second stage refers to the maternal systemic manifestations characterized by inflammatory, metabolic, and thrombotic responses that converge to alter vascular function, which can result in multiorgan damage (***Jeyabalan A,2013***).

One of the most striking physiologic changes is intense systemic vasospasm, which is responsible for decreased perfusion of virtually all organ systems. Perfusion also is diminished because of vascular hemoconcentration and third spacing of intravascular fluids. In addition, preeclampsia is accompanied by an exaggerated inflammatory response and inappropriate endothelial activation. Activation of the coagulation cascade and resultant microthrombi formation further compromise blood flow to organs (***Lana K,2004***).

In spite of several research, causes for the development of the disease and the economical best marker for diagnosis of HDP remains vague till date. Several studies have documented preeclampsia as a disease of placenta and the best mode to treat is to deliver the placenta (***Reeta et al.,2014)***.

2.1.3 Classification of Hypertension Disorder of Pregnancy

Diagnosis becomes less difficult if physicians understand where preeclampsia "fits" into the hypertensive disorders of pregnancy. These disorders include chronic hypertension, preeclampsia-eclampsia, preeclampsia superimposed on chronic hypertension, and gestational hypertension (***Lana K,2004***).

2.1.3.1 Chronic hypertension

Is defined by elevated blood pressure that predates the pregnancy, is documented before 20 weeks of gestation, or is present 12 weeks after delivery (***Lana K,2004. Reeta et al.,2014***).

2.1.3.2 Pre-eclampsia

Is defined by elevated blood pressure and proteinuria that occur after 20 weeks of gestation. Eclampsia, a severe complication of preeclampsia, is the new onset of seizures in a woman with preeclampsia. Eclamptic seizures are relatively rare and occur in less than 1 percent of women with preeclampsia (***Lana K,2004***).

2.1.3.3 Preeclampsia superimposed hypertension

Is characterized by new-onset proteinuria (or by a sudden increase in the protein level if proteinuria already is present), an acute increase in the level of hypertension

(assuming proteinuria already exists), or development of the HELLP (hemolysis, elevated liver enzymes, low platelet count) syndrome (***Lana K,2004. Reeta et al.,2014***).

2.1.3.4 Gestational hypertension

Is diagnosed when elevated blood pressure without proteinuria develops after 20 weeks of gestation and blood pressure returns to normal within 12 weeks after delivery. One fourth of women with gestational hypertension develop proteinuria and thus progress to preeclampsia (***Lana K, 2004. Reeta et al., 2014***).

2.1.4 Symptoms and Signs of Preeclampsia

Although the symptoms and signs of preeclampsia occur along a continuum, the syndrome is often categorized as mild or severe to communicate the severity of disease and the management approach. Preeclampsia is considered severe when at least one of the following is present in addition to the defining blood pressure and proteinuria criteria : 1) systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥110 mmHg; 2) urinary protein excretion of =5 g in a 24-h collection; 3) neurologic disturbances (visual changes, headache, seizures, coma); 4) pulmonary edema; 5) hepatic dysfunction (elevated liver transaminases or epigastric pain); 6) renal compromise (oliguria or elevated serum creatinine concentration; creatinine ≥1.2 mg/dL is considered abnormal in women without a history of renal disease); 7) thrombocytopenia; 8) placental abruption, fetal growth restriction, or oligohydramnios. Eclampsia refers to seizures in a preeclamptic woman that cannot be attributed to other causes. The hypertensive disorder referred to as HELLP syndrome is defined by the presence of hemolysis (H), elevated liver transaminases (EL), and low platelet counts (LP). This may or may not occur in the presence of hypertension or proteinuria, but it is considered to be along the spectrum of preeclampsia (***Jeyabalan A,2013***).

2.1.5 Diagnosis of Preeclampsia

Diagnostic criteria for preeclampsia includenew onset of elevated blood pressure and proteinuria weeks of gestation. Features such as edema and blood 20 after

s baseline no longe'pressure elevation above the patient are diagnostic criteria.Severe preeclampsia is indicated by more substantial bloodpressure elevations and a greater degree of ther features of severe preproteinuria. Oeclampsia include oliguria, cerebral or visual disturbances, and pulmonary edema or cyanosis (***Lana K,2004***).

Also pre-eclampsia (PE) was defined as a new-onset persistent blood pressure (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg) and proteinuria (based on a 24 h urine collection with a total protein excretion > 300 mg or a urinary spot measurement of protein-to-creatinine ratio > 0.3) or, in the absence of proteinuria, new onset hypertension with the new onset of any of the following: thrombocytopenia (platelets < 100 × 109/L), renal insufficiency (serum creatinine > 1.1 mg/dL or doubling creatinine in the absence of other renal disease), impaired liver function (elevated blood concentrations of liver transaminases to twice normal concentration), pulmonary edema, and unexplained new-onset headache unresponsive to medications or visual symptoms after 20 weeks of gestation, according to the American College of Obstetricians and Gynecologists (ACOG 2018).(***Chaparro et al.,2020)***.

Several biochemical parameters: serum albumin, total protein, iron, iron binding capacity, hemoglobin, ferritin, uric acid, Magnesium, aminotransferases, total ALP, PLAP and urinary protein had been cited in the past which find wide use in predicting pregnancy hypertension. In recent years, however certain novel biochemical substances such as prostaglandins, Inhibin, Cell Adhesion Molecules have been shown to possess clinical relevance in monitoring the course and outcome of hypertensive disorders of pregnancy. There are studies showing a raised serum uric acid level in HDP compared to normal pregnant women. Increase in uric acid is considered as predictor of severity of disease (***Reeta et al.,2014)***.

2.1.6 Laboratory Evaluation of Preeclampsia

There currently is no single reliable, cost-effective screening test for preeclampsia. the serum uric acid level once was used as an indicator of preeclampsia but has been found to lack sensitivity and specificity as a diagnostic tool. However, an elevated serum uric acid level may be of some use in identifying pregnant women with chronic hypertension who have an increased likelihood of having superimposed preeclampsia. A baseline laboratory evaluation should be performed early in pregnancy in women who are at high risk for preeclampsia. Tests should include a hepatic enzyme level, a platelet count, a serum creatinine level, and a 12- to 24-hour urine collection for total protein measurement. Once the diagnosis of preeclampsia has been made, an expanded set of laboratory tests should be performed. In women who have preeclampsia with no suspected progression, all laboratory tests should be conducted weekly. If progression of eclampsia is suspected, the tests should be repeated more frequently (***Lana K,2004***).

Elevated activities of hepatic enzymes are indicative of hepatocellular injury in PE cases. ALP is a membrane associate ubiquitous enzyme involved in the transport of phosphate and carbohydrate across the trophoblast cell membranes which is imperative for placental metabolism and later to fetus; raised activity of ALP has been reported in normal pregnancy than nonpregnant women which attribute to its placental isozymes. About 2-fold increased ALP in PE groups indicative of placental dysfunction and may be a warning to impending fetal health (***Jigeesha et al.,2021***).

Maternal soluble endoglin (sEng) and soluble fms-like tyrosine kinase-1 (sFlt1) may be possible. markers in early gestational age for prediction of PE There are also studies suggesting that MPV was significantlyhigher in preeclamptic women than in healthy pregnantwomen and thus may be a promising biomarker for the PE detection and follow up . Systemic immune inflammation indices such as lymphocyte ratio (NLR)-neutrophil and monocyte-lymphocyte ratio (MLR) are also

reported to beeffective in clinical assessment, disease severity evaluation, and prognosis evaluation of PE. Changes of serum traceelements like Ca, Cu, and Mg are reported to be associatedwith PE, while serum ceruloplasmin and soluble LIGHTare reported to have predictive value in PE (***Duan et al.,2020***).

2.1.7 Treatment of Preeclampsia

Delivery remains the ultimate treatment for preeclampsia. Although maternal and fetal risks must be weighed in determining the timing of delivery, clear indications for delivery exist. When possible, vaginal delivery is preferable to avoid the added physiologic stressors of cesarean delivery. If cesarean delivery must be used, regional anesthesia is preferred because it carries less maternal risk (***Lana K,2004***). Since delivery may become necessary rapidly, corticosteroids for fetal lung maturity should be considered at the diagnosis of severe early onset preeclampsia. Women meeting the criteria for delivery would normally also merit treatment with magnesium sulfate. Women who meet the criteria for the diagnosis of severe preeclampsia or have significant signs and symptoms of impending eclampsia (severe headache, clonus, neurological impairment) should be considered for treatment with magnesium sulfate for eclampsia prophylaxis. Magnesium sulfate is associated with a 50%–67% reduction in the risk of seizures, a reduction in the risk of maternal death, and may have some benefit to the baby. The standard protocol is as described in the Collaborative Eclampsia Trial, a 4 g loading dose with a maintenance infusion of 1 g/h. Treatment with magnesium sulfate requires at least level 2 care, with close monitoring, observation of urine output, respiratory rate, and deep tendon reflexes. After 34 weeks, the decision to deliver should be based on the condition of the mother and baby and any identifiable risk factors for progression of diseases (***Townsend et al.,2016***).

Brief communication cites evidence that might suggest a moderately effective treatment for preeclampsia. The therapy consists of having the gravida with preeclampsia stay in a room provided with FACE (Free Air Carbon-dioxide Enrichment). No containment (such as in greenhouses) is required with FACE

systems. Evidence is cited which suggests that the therapy might reduce the incidence of preeclampsia by perhaps 10–30% without much harm to the fetus (***Brown D,2020)*.**

2.1.8 Prevention of Preeclampsia

There currently are no well-established measures for preventing preeclampsia. Both low-dose aspirin therapy and daily calcium supplementation have been studied as preventive measures but have not been shown to be beneficial in the general pregnant population and are not recommended for primary prevention of preeclampsia. Some evidence does support the use of low-dose aspirin therapy and daily calcium supplementation in certain high-risk women. Calcium supplementation has been shown to produce modest blood pressure reductions in pregnant women who are at above-average risk for hypertensive disorders of pregnancy and in pregnant women with low dietary calcium intake. An optimum calcium dosage for these women has not been established (***Atallah et al.,2018. Lana K,2004)***.

Low-dose aspirin therapy (100 mg per day or less) has been shown to reduce the incidence of preeclampsia in women who were found to have an abnormal uterine artery on Doppler ultrasound examination performed in the second trimester. Research on the use of antioxidants in the prevention of preeclampsia is promising. However, further study is needed, and antioxidant therapy currently is not recommended. Although preeclampsia is not preventable, many deaths from the disorder can be prevented. Women who do not receive prenatal care are seven times more likely to die from complications related to preeclampsia, eclampsia than women who receive some level of prenatal care. Some studies indicate that preeclampsia-related fatalities occur three times more often in black women than in white women. Although the precise reasons for the racial differences remain elusive, the differences may be indicative of disparities in health status, as well as access to and quality of prenatal care. To decrease preeclampsia-related mortality, appropriate prenatal care must be available to all women. Early detection, careful monitoring,

and treatment of preeclampsia are crucial in preventing mortality related to this disorder (***Mackay et al.,2001. Lana K,2004***).

2.2 Enzyme

2.2.1 Alkaline Phosphatase

Alkaline phosphatase (ALP) belongs to a group of enzymes that catalyze the hydrolysis of various phosphomonoesters at an alkaline PH. Consequently, ALP is a nonspecific enzyme capable of reacting with many different substrates. Specifically, ALP functions to liberate inorganic phosphate from an organic phosphate ester with the concomitant production of an alcohol. The optimal pH for the reaction is 9.0 to 10.0, but optimal pH varies with the substrate used. The enzyme requires Mg2 as an activator. (***Bishop et al.,2010***).

2.2.2 Tissue Source of ALP

ALP activity is present on cell surfaces in most human tissue. The highest concentrations are found in the intestine, liver, bone, spleen, placenta, and kidney. In the liver, the enzyme is located on both sinusoidal and bile canalicular membranes; activity in bone is confined to the osteoblasts, those cells involved in the production of bone matrix. The specific location of the enzyme within this tissue accounts for the more predominant elevations in certain disorders. (***Bishop et al.,2010***).

Placenta ALP (PALP) is a membrane-bound glycoprotein, expressed by the maternal microvillous membrane of the syncytiotrophoblast. The concentration of PLAP in maternal blood increases throughout gestation in normal pregnancy and has been implicated in regulating fetal/maternal metabolism, the transport of nutrients, and placental differentiation (***Chaparro et al.,2020***).

2.2.3 Diagnosis Significance of ALP

Elevations of ALP are of most diagnostic significance in the evaluation of hepatobiliary and bone disorders. In hepatobiliary disorders, elevations are more predominant in obstructive conditions than in hepatocellular disorders; in bone disorders, elevations are observed when there is involvement of osteoblasts. In

biliary tract obstruction, ALP levels range from 3 to 10 times ULN. Increases are primarily a result of increased synthesis of the enzyme induced by cholestasis. In contrast, hepatocellular disorders, such as hepatitis and cirrhosis, show only slight increases, usually less than three times ULN. Because of the degree of overlap of ALP elevations that occurs in the various liver disorders, a single elevated ALP level is difficult to interpret. It assumes more diagnostic significance when evaluated along with other tests of hepatic function. Elevated ALP levels may be observed in various bone disorders. Perhaps the highest elevations of ALP activity occur in Paget's disease (osteitis deformans). Other bone disorders include osteomalacia, rickets, hyperparathyroidism, and osteogenic sarcoma. In addition, increased levels are observed in healing bone fractures and during periods of physiologic bone growth. In normal pregnancy, increased ALP activity, averaging approximately 1,5 times ULN, can be detected between weeks 16 and 20. ALP activity increases and persists until the onset of labor. Activity then returns to normal within 3 to 6 days. Elevations also may be seen in complications of pregnancy such as hypertension, preeclampsia, and eclampsia, as well as in threatened abortion. ALP levels are significantly decreased in the inherited condition of hypophosphatasia. Subnormal activity is a result of the absence of the bone isoenzyme and results in inadequate bone calcification. ALP exists as a number of isoenzymes, which have been studied by a variety of techniques. The major isoenzymes, which are found in the serum and have been most extensively studied, are those derived from the liver, bone, intestine, and placenta. Electrophoresis is considered the most useful single technique for ALP isoenzyme analysis. However, because there may still be some degree of overlap between the fractions, electrophoresis in combination with another separation technique may provide the most reliable information. A direct immunochemical method for the measurement of bone-related ALP is now available; this has made ALP electrophoresis unnecessary in most cases. The liver fraction migrates the fastest, followed by bone, placental, and intestinal fractions. Because of the similarity between liver and bone phosphatases, there often is not a

clear separation between them. Quantitation with use of a densitometer is sometimes difficult because of the overlap between the two peaks. The liver isoenzyme can actually be divided into two fractions—the major liver band and a smaller fraction called fast liver, or a1 liver, which migrates anodal to the major band and corresponds to the a1 fraction of protein electrophoresis. When total ALP levels are increased, the major liver fraction is the most frequently elevated. Many hepatobiliary conditions cause elevations of this fraction, usually early in the course of the disease. The fast-liver fraction has been reported in metastatic carcinoma of the liver, as well as in other hepatobiliary diseases. Its presence is regarded as a valuable indicator of obstructive liver disease. However, it is occasionally present in the absence of any detectable disease state. The bone isoenzyme increases due to osteoblastic activity and is normally elevated in children during periods of growth and in adults older than age 50. In these cases, an elevated ALP level may be difficult to interpret. The presence of intestinal ALP isoenzyme in serum depends on the blood group and secretor status of the individual. Individuals who have B or O blood group and are secretors are more likely to have this fraction. Apparently, intestinal ALP is bound by erythrocytes of group A. Furthermore, in these individuals, increases in intestinal ALP occur after consumption of a fatty meal. Intestinal ALP may increase in several disorders, such as diseases of the digestive tract and cirrhosis. Increased levels are also found in patients undergoing chronic hemodialysis (***Bishop et al.,2010***).

Difference in heat stability is the basis of a second approach used to identify the isoenzyme source of an elevated ALP. Typically, ALP activity is measured before and after heating the serum at 56°C for 10 minutes. If the residual activity after heating is less than 20% of the total activity before heating, then the ALP elevation is assumed to be a result of bone phosphatase. If greater than 20% of the activity remains, the elevation is probably a result of liver phosphatase. These results are based on the finding that placental ALP is the most heat stable of the four major fractions, followed by intestinal, liver, and bone fractions in decreasing order of heat

stability. Placental ALP will resist heat denaturation at 65°C for 30 minutes. Heat inactivation is an imprecise method for differentiation because inactivation depends on many factors, such as correct temperature control, timing, and analytic methods sensitive enough to detect small amounts of residual ALP activity. In addition, there is some degree of overlap between heat inactivation of liver and bone fractions in both liver and bone diseases (***Bishop et al.,2010***).

A third approach to identification of ALP isoenzymes is based on selective chemical inhibition. Phenylalanine is one of several inhibitors that have been used. Phenylalanine inhibits intestinal and placental ALP to a much greater extent than liver and bone ALP. With phenylalanine use, however, it is impossible to differentiate placental from intestinal ALP or liver from bone ALP. In addition to the four major ALP isoenzyme fractions, certain abnormal fractions are associated with neoplasms. The most frequently seen are the Regan and Nagao isoenzymes. They have been referred to as carcinoplacental alkaline phosphatases because of their similarities to the placental isoenzyme. The frequency of occurrence ranges from 3% to 15% in cancer patients. The Regan isoenzyme has been characterized as an example of an ectopic production of an enzyme by malignant tissue. It has been detected in various carcinomas, such as lung, breast, ovarian, and colon, with the highest incidences in ovarian and gynecologic cancers. Because of its low incidence in cancer patients, diagnosis of malignancy is rarely based on its presence. It is, however, useful in monitoring the effects of therapy because it will disappear on successful treatment. The Regan isoenzyme migrates to the same position as the bone fraction and is the most heat stable of all ALP isoenzymes, resisting denaturation at 65°C for 30 minutes. Its activity is inhibited by phenylalanine. The Nagao isoenzyme may be considered a variant of the Regan isoenzyme. Its electrophoretic, heat-stability, and phenylalanine-inhibition properties are identical to those of the Regan fraction. However, Nagao also can be inhibited by L-leucine.

Its presence has been detected in metastatic carcinoma of pleural surfaces and in adenocarcinoma of the pancreas and bile duct (***Bishop et al.,2010***).

Chapter Three

Materials and Methods

3. Materials and Methods

3.1 Study Design, Area and Period

This cross sectional comparative hospital based study conducted in Khartoum state at Omdurman Maternity Hospital from March to June 2022.

3.2 Ethical Consideration

The study protocol was approved by institutional ethic committee of Sudan university for science and technology , before initiation of study an informed consent was obtained from each participant before enrolling method (Appendix Ⅰ).

3.3 Study Subjects

Sudanese pregnant women diagnosed with preeclampsia were enrolled in this study as case group and apparently healthy pregnant women were included as control group.

3.4 Inclusion Criteria

Pregnant women diagnosed with preeclampsia which blood pressure ≥140/90.

3.5 Exclusions Criteria

Pregnant women with Jaundice, chronic liver disorders, and other pregnancy associated disorder and complicated pregnancies were excluded.

3.6 Sampling Technique

The study sample selected by haphazard (convenience) samples.

3.7 Data Collections

using structured questionnaire Data collected (Appendix Ⅱ)by direct interview and data from sera analysis for total ALP and PLAP estimated and recorded in the same forms.

3.8 Blood Sampling and Process

Five ml of blood sample (from both normal and hypertensive mothers) was collected in a plain vacutainer. The individual samples were centrifuged at 1200 rpm for 10 minutes and the separated serum was used for the analysis of Alkaline Phosphatase.

Before analysis of PALP took 0.5 ml of sera samples were added into small thin-walled glass tubes placed in thermostatically controlled water bath stabilized at 65 °C. the water level was at least 3 cm above the samples exactly following 30 minutes. The analysis carried out on the same day within four hours so as to minimize the inactivation of ALP by denaturation. The biochemical assays were carried out using procedures approved by the International Federation of Clinical Chemistry (IFCC).

3.9 Estimation of Serum ALP

3.9.1 Principle of Method

p-Nitrophenyl phosphate (colorless) is hydrolyzed to p-nitrophenol (yellow), and the increase in absorbance at 405 nm, which is directly proportional to ALP activity, was measured (**Bishop *et al*.,2010).**

3.9.2 Reagent Composition, Storage, Procedure

Appendix Ⅲ.

3.10 Quality Control

To ensure adequate quality control and to verify the performance of measurement procedures were monitored by using of biochemistry control serum normal level 1 and control serum abnormal level 2.

3.11 Statistical Study

All data analyzed using statistical package for social science (SPSS), the results express as mean, ±SD. Ites-ndependent Tt used to compare between mean concentration of enzymes in patients versus the control group, and Pearson correlation to correlate between study parameter and study variable, P-value <0.05 consider as significant. Also, Receiver Operator characteristic Curve (ROC) analysis was used to detect sensitivity, specificity and cutoff of study parameter as predictor marker of preeclampsia in pregnant women.

Chapter Four

Results

4.Results

The study included 100 Sudanese pregnant women aged between (15-45) years old, of them 50 pregnant women with preeclampsia and 50 healthed apparently pregnant women as control, conducted in Omdurman Maternity Hospital form March to June 2022 Khartoum- Sudan.

The result showed study population (Mean±SD) with study variables (Age range from 15- 45 years old with mean ±SD (27.7±6.02) years. Number of gestational range from 1-10 times with mean ±SD (3.04±2.15) times. Gestational Age range from 6-9 months with mean±SD (8.7±0.74) months. Number of children death range from 0-5 children with mean ±SD (0.53 ±0.90). Number of children alive that range from 0-9 children with mean±SD (1.46 ± 1.8). In (Table 4.1).

The result showed that increase the risk to disease (preeclampsia) among (21-30) years old pregnant women with percentage of 44% in compared to other age range among participants (Table 4.2).

Study showed the frequency of Children death and Children a live among case (36%), (52%) in compared to control group (34%), (62%) respectively. (Figure 4.1).

Also, study showed the frequency of normal delivery and caesarean section delivery among case (12%), (38%) in compared to control group (36%), (15%) respectively. (Figure 4.2).

The study found that the percentage of diabetes mellitus among case only (5%) when compare to control (Figure 4.3).

In compared between preeclamptic pregnant women and normal pregnant women, Study found that insignificant increase of ALP with (P.value=0.33); but the PALP and Ratio significant increase with (P.value 0.005 ,0.000) respectively, in (Table 4.3).

Moreover, in correlation of total alkaline phosphatase (ALP), placenta alkaline phosphatase (PALP) and ratio with age the result showed insignificant correlation with them (r= -0.131, -0.168, -0.096. P.value=0.363, 0.456, 0.505), otherwise in correlated with SBP there was insignificant correlation (r= 0.029, 0.039, 0.042. P.value= 0.840, 0.788, 0.773), also in correlated with DBP found that insignificant correlation (r= 0.019, 0.048, 0.087. P.value= 0.898, 0.742, 0.550), finally in correlated with gravida result showed insignificant correlation (r= 0.065, 0.028, -0.102. P.value=0.652, 0.844, 0.479) for all parameters respectively. show by (Table 4.4).

In receiver operating characteristics curve (ROC) the PALPshowed an optimum cut off at 133 with 56% sensitivity and 72% specificity , moreover theALP showed an optimum cut off at 212 with 56% sensitivity and 60% specificity; while the ratio showed an optimum cut off at 0.589 with 66% sensitivity and 64% specificity in patients with preeclampsia (Table 4.5, Figure 4.4).

Table (4.1): Demographical data of Study group.

Variables	N	Minimum	Maximum	Mean	Std. Deviation
Age / year	100	15	45	27.7	6.02
Number of gestational	100	1	10	3.04	2.15
Gestational age / Month	100	6	9	8.7	0.74
Number of children death	100	0	5	0.53	0.90
Number of children alive	100	0	9	1.46	1.8

Table (4.2): Distribution of Age group among participants.

Age group	**Case Group N (%)**	**Control Group N (%)**
less or equal to 20 years	6 (12%)	6 (12%)
21 -30 years	22 (44%)	27 (54%)
31 - 40 years	21 (42%)	16 (32%)
above 40 years	1 (2%)	1 (2%)
Total	50 (100%)	50 (100%)

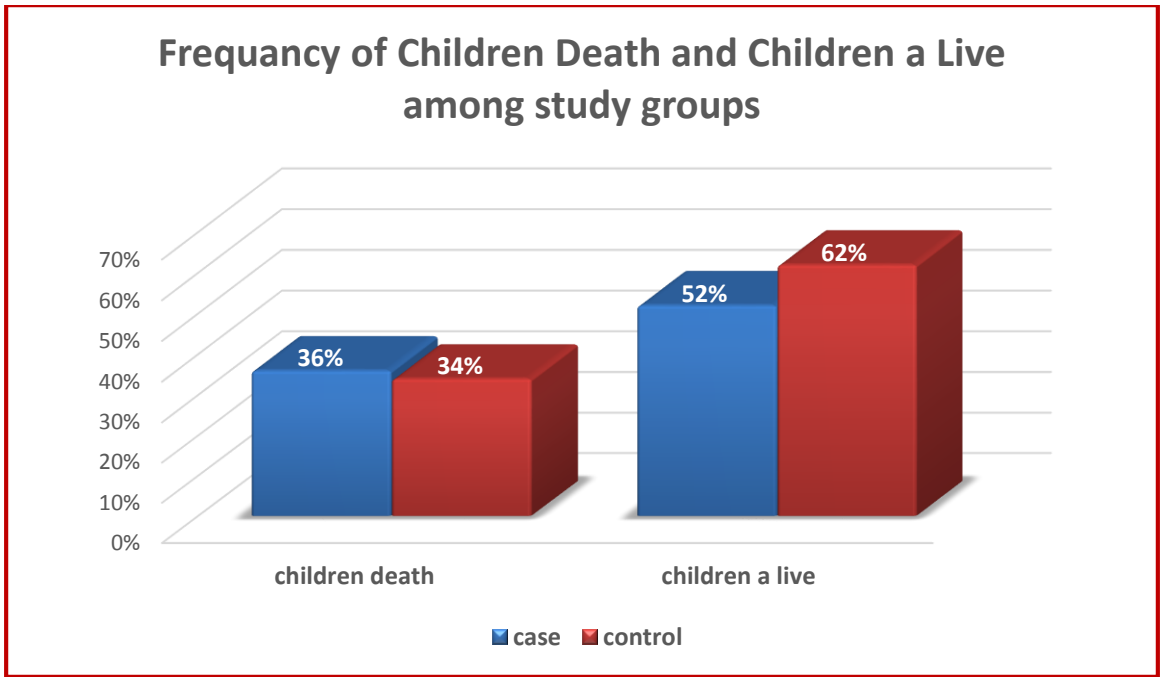

Figure (4.1): Frequency of Children death and Children a live among study groups.

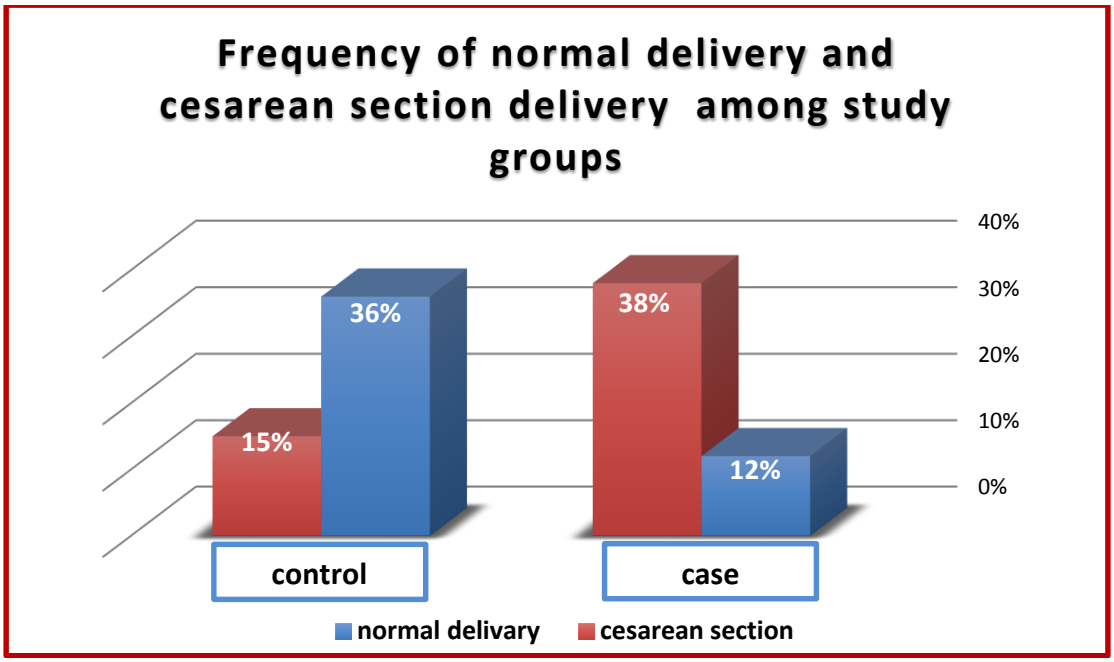

Figure (4.2): Frequency of normal delivery and cesarean section delivery among study groups.

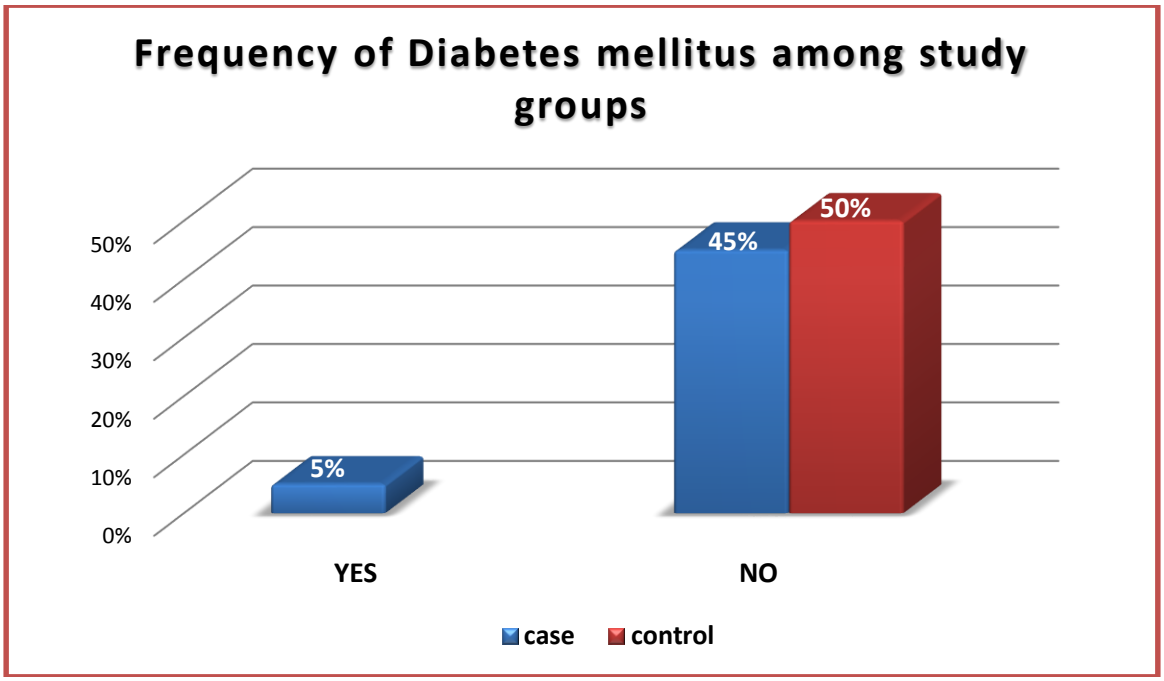

Figure (4.3): Frequency of Diabetes mellitus among study groups.

Table (4.3): Comparison of Total and Placental Alkaline Phosphatase in Case and Control Group.

Parameters	**Case Group (N= 50)**	**Control Group (N= 50)**	**P- value**
ALP IU/L	221.8 ± 76.4	204.06 ± 93.2	0.33
PALP IU/L	151.5 ± 76.1	111.1 ± 65.4	0.005 *
Ratio PALP/ALP	0.66 ± 0.2	0.52 ± 0.1	0.000 *

- Independent sample t-test was used to compare between means.
- P-value considered significant at 0.05.

Table (4.4): Correlation of Total, Placental Alkaline Phosphatase and Ratio with Age, Systolic (SBP) and Diastolic (DBP) Blood Pressure and Gravida.

		Age	SBP	DBP	Gravida
ALP	**r**	-0.131	0.029	0.019	0.065
	P	0.363	0.840	0.898	0.652
PALP	**r**	-0.108	0.039	0.048	0.028
	P	0.456	0.788	0.742	0.844
Ratio PALP/ALP	**r**	-0.096	0.042	0.087	-0.102
	P	0.505	0.773	0.550	0.479

- Pearson used to correlate between study parameter and study variable.
- P- value considered significant at 0.05.

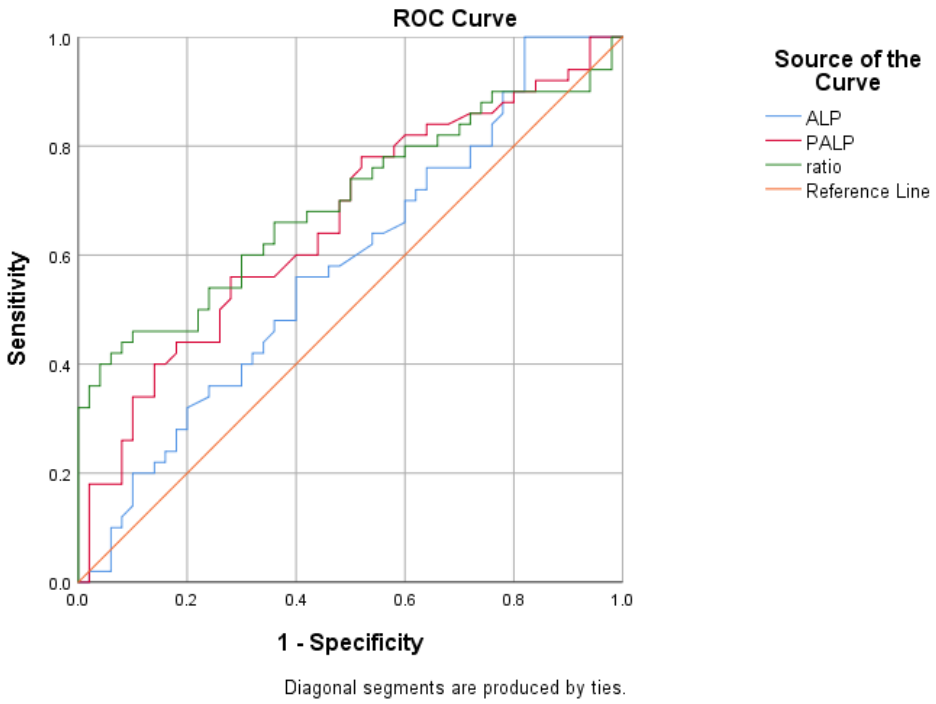

Figure (4.4): Receiver operating characteristics curve of total, placental ALP and ratio.

Table (4.5): Receiver operating characteristics curve of total, placental ALP and ratio.

	Confidence Interval	AUC	p-value	%Sensitivity	%Specificity	Cut off
ALP	0.470 - 0.694	0.582	0.159	56%	60%	212
PALP	0.552- 0.766	0.659	0.006	56%	72%	133
Ratio PALP/ALP	0.591-0.800	0.695	0.001	66%	64%	0.589

Chapter Five

Discussion , Conclusion , Recommendations

5. Discussion, conclusion and recommendations

5.1 Discussion

Preeclampsia, one of the most common hypertensive disorders during pregnancy complicates 2–8% of pregnancies worldwide, characterized by impaired trophoblastic penetration of decidua, less uteroplacental blood perfusion, inflammation, apoptosis, structural damage of fetus and increased risks of future cardiovascular, renal, and neurological complications (***Weissgerber and Mudd,2015. Bokslag et al.,2016***).

The study showed insignificant increase in the level of ALP in case compared to control groups. This finding disagreed with study done by Munazza and his group which finding that the levels of ALP were significantly higher in hypertensive preeclampsia cases than in normotensive controls (P-value <0.001) (***munazza et al.,2011)***. This maybe due to that patient may had mild disease so the ALP may not elevated enough to be significant related to preeclampsia, or due to different in genetic makeup.

Also, study showed significant increase in serum PALP in case compared to control group. This finding agreed with study done by Mishra and his colleagues which finding that about 2-fold increased ALP in PE groups indicative of placental dysfunction and may be a warning to impending fetal health (***Mishra et al.,2021).*** Moreover, study by Hutchinson and his group found that the elevated levels of serum PALP in hypertensive pregnant women may be attributed to placental dysfunction, which results in increased serum levels of these enzymes. Shedding of syncytiotrophoblast into the maternal circulation is a normal part of pregnancy, but is increased during pre-eclampsia. In pre-eclampsia, this process of syncytio-trophoblast renewal is overactive and complicated by necrosis and apo necrosis of the syncytio-trophoblast particles (***Hutchinson et al.,2009).***

Also, study showed significant increase in ratio PALP/ALP in case compared to control group. This finding agree with study done by Rajagambeeram and his colleagues which found that the ratio of PLAP/ALP was significantly higher in hypertensive disorders of pregnancy with p-values of <0.001.(***Rajagambeeram et al.,2014).***

In this study showed insignificant correlation between ALP and the age, Systolic blood pressure (SBP), Diastolic blood pressure (DBP), and gravida. Also, study showed insignificant correlation between PALP with age, Systolic blood pressure (SBP), Diastolic blood pressure (DBP), and gravida. In addition, this study showed insignificant correlation between ratio PALP/ALP and the age, Systolic blood pressure (SBP), Diastolic blood pressure (DBP), and gravida. Those findings disagree with study by Rajagambeeram and his group which found that there was significant correlation among ALP and DBP and there is a good positive correlation between PLAP and blood pressure. (***Rajagambeeram et al.,2014).*** Moreover, disagree with study by Duan and his team which found that women conceive at older age are at higher risk of developing PE with significant difference in level ALP between the control group and mild, sever PE group. (***Duan et al,2020).*** Also disagree with study by Prashant and his colleagues which found that measurable levels of ALP appear in maternal serum by the end of first trimester and increases progressively with gestational age (***Prashant et al, 2016***). Also disagree with study by Ferianec and Linhartova which found that extreme elevation of alkaline phosphatase in the 3rd trimester of gestation may be an important marker of placental insufficiency. (***Ferianec V and Linhartova L, 2011).*** The different in the results may be due to change in country that study conducted in it with change in ethnicity. Also, the most women in study in younger age and in last month of third trimester so level of enzyme elevated stable.

However, the use of PALP and ratio as prognostic parameter for preeclampsia with other biochemical test, in small and medium health center provide early detection

and treatment to preeclampsia pregnant women. these confirmed to study of Rajagambeeram and his group which showed a better and practical approach towards the assessment of hypertensive disorders of pregnancy would be to utilize heat stable ALP isoenzyme (PALP) and PALP/ALP ratio as an adjuvant marker in the armamentarium of biochemical tests, especially since the same is simple to assay and reliable as well as economical and sensitive (***Rajagambeeram et al.,2014).***

5.2 Conclusion

The study concluded that Sudanese women with preeclampsia had elevated serum PALP and ratio of PALP/TALP. Moreover, PALP and ratio of PALP/TALP can be used as predictive marker of preeclampsia in pregnancy.

5.3 Recommendations

1. Periodic estimation of TALP, PALP and their ratio in pregnant women to assess their diagnostic utility as reliable and economical biochemical markers of pregnancy induced hypertension.

2. Further studies about ALP and it is isoenzyme in preeclampsia in different state in Sudan.

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Appendices

Appendix Ⅰ: Arabic version of formal consent

بسم الله الرحمن الرحيم

أذن لاخذ معلومات بغرض البحث

بحث لدراسة:

الفائدة من ALP في المصل والمشيمة كعلامة تنبؤيه لتسمم الحمل لدى النساء السودانيات الحوامل بولايه الخرطوم.

بيان الباحث:

انا اطلب منك المشاركه في دراسه بحثيه الغرض من هذا الفورم ان نعطيك المعلومات التي ستحتاجها لتساعدك على ان تقرر ما أذا كنت ستشارك في الدراسه ام لا. يمكنك طرح اسئله حول الغرض من البحث، حقوقك كمتطوع ،او اي شي اخر غير واضح بالنسبه لك بخصوص البحث او هذا الفورم.عندما نجيبك على اسئلتك يمكنك ان تقررما اذا كنت ستشارك في الدراسه ام لا.

الغرض من الدراسه:

الفائدة من ALP في المصل والمشيمة كعلامة تنبؤيه لتسمم الحمل لدى النساء السودانيات الحوامل بولايه الخرطوم. مما يساعد الاطباء على معرفه وتطوير تشخيص وعلاج مرضاهم وايجاد الحلول المناسبه للوقايه من هذا المرض.

اجراءات الدراسه:

اذا قررت المشاركه في هذه الدراسه؛ سأجري معك مقابله عن طريق استبيان مكتوب يتكون من عدة اسئله سأملأه بنفسي. سأسألك اسئله متعلقه بالدراسه، هذه المقابله ستستغرق حوالي 5 دقايق لست ملزم بالأجابه عن كل الاسئله سأدون اجاباتك وسأستخدمها في الدراسه. ونود ان نشير ان المشاركه في البحث طوعيه وان رفضك للمشاركه في البحث لايفقدك الحق في اي من فوائد الدراسه.

- هذه المعلومات التي اخذت منك ستكون سريه. اذا تم نشر او عرض نتائج هذه الدراسه فاننا لن نستخدم الاسم مع النتائج المنشوره او المعروضه.
- التوقيع/ .....
- التاريخ/.....

Appendix Ⅱ

Sudan University of Science and Technology

College Of Graduate Studies

Utility of Serum and placental ALP as marker for preeclampsia among Sudanese females

Questionnaire number (……..)

A. General Information:

- Patient name ……
- Age …… years

B. Clinical Information:

- Gestational Age.....
- Number of Children death.....
- Number of children a live.....
- Blood pressure …….
- Number of gestation.....
- History of Chronic diseases

1\ DM: Yes( ) No ( )

2\ Hypertension: Yes ( ) No ( )

C. Delivery Information:

- Normal delivery ( )
- Caesarean section ( )

D. Investigation:

- Total ALP …… U/L
- PALP …… U/L

Appendix Ⅲ

COD 11592 50 mL	COD 11593 200 mL	COD 11598 500 mL
STORE AT 2-8°C		
Reagents for measurement of ALP concentration Only for *in vitro* use in the clinical laboratory		

PRINCIPLE OF THE METHOD

Alkaline phosphatase (ALP) catalyzes in alkaline medium the transfer of the phosphate group from 4-nitrophenylphosphate to 2-amino-2-methyl-1-propanol (AMP), liberating 4-nitrophenol. The catalytic concentration is determined from the rate of 4-nitrophenol formation, measured at 405 nm[1].

4 – Nitrophenylphosphate + AMP $\xrightarrow{ALP}$ AMP – phosphate + 4 – Nitrophenol

CONTENTS

	COD 11592	COD 11593	COD 11598
A. Reagent	1 x 40 mL	1 x 160 mL	4 x 100 mL
B. Reagent	1 x 10 mL	1 x 40 mL	2 x 50 mL

COMPOSITION

A. Reagent: 2-Amino-2-methyl-1-propanol 0.4 mol/L, zinc sulfate 1.2 mmol/L, N-hydroxyethylethylenediaminetriacetic acid 2.5 mmol/L, magnesium acetate 2.5 mmol/L, pH 10.4.

B. Reagent: 4-Nitrophenylphosphate 60 mmol/L.

STORAGE

Store at 2-8°C.

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

Indications of deterioration:

- Reagents: Presence of particulate material, turbidity, absorbance of the blank over 1.200 at 405 nm (1 cm cuvette).

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents. Safety data sheet available for professional user on request. Disposal of all waste material should be in accordance with local guidelines. Any serious incident that might occur in relation to the device shall be reported to BioSystems S.A.

REAGENT PREPARATION

Working Reagent:

- Cod. 11592 and 11593: Transfer the contents of one Reagent B vial into a Reagent A bottle. *Mix gently.* Other volumes can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B. Stable for 2 months at 2-8°C.
- Cod. 11598: Transfer 25 mL of one Reagent B vial into a Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B. Stable for 2 months at 2-8°C.

ADDITIONAL EQUIPMENT

- Analyzer, spectrophotometer or photometer with cell holder thermostatable at 37°C and able to read at 405 nm.
- Cuvettes with 1 cm light path.

SAMPLES

Serum and plasma collected by standard procedures.

Alkaline phosphatase in serum or plasma is stable for 7 days at 2-8°C. Heparin may be used as anticoagulant.

PROCEDURE

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

Working Reagent	1.0 mL
Sample	20 µL

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

CALCULATIONS

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

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

The molar absorbance (ε) of 4-nitrophenol at 405 nm is 18450, the lightpath (l) is 1 cm, the total reaction volume (Vt) is 1.02, the sample volume (Vs) is 0.02, and 1 U/L are 0.0166 µkat/L. The following formulas are deduced for the calculation of the catalytic concentration:

ΔA/min	x 2754 = U/L x 46.08 = µkat/L

ALKALINE PHOSPHATASE (ALP) - AMP

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ALKALINE PHOSPHATASE (ALP) - AMP
2-AMINO-2-METHYL-1-PROPANOL BUFFER (IFCC)

REFERENCE VALUES

Reaction temperature	men	women
37°C, up to[2]	115 U/L = 1.92 µkat/L	105 U/L = 1.75 µkat/L

Concentrations in growing children are higher and highly variable. These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

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

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

METROLOGICAL CHARACTERISTICS

- Detection limit: 1.0 U/L = 0.017 µkat/L
- Linearity limit: 1200 U/L = 20 µkat/L. For higher values dilute sample 1/2 with distilled water and repeat measurement.
- Repeatibility (within run):

Mean Concentration	CV	n
61 U/L = 1.02 µkat/L	1.0 %	20
244 U/L = 4.07 µkat/L	0.7 %	20

- Reproducibility (run to run):

Mean Concentration	CV	n
61 U/L = 1.02 µkat/L	3.4 %	25
244 U/L = 4.07 µkat/L	1.1 %	25

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.
- Interferences: Lipemia (triglycerides < 10 g/L) and bilirubin (< 20 mg/dL) do not interfere. Hemoglobin (> 2.5 g/L) interfere. Other drugs and substances may interfere[3].

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

DIAGNOSTIC CHARACTERISTICS

Alkaline phosphatase catalyzes the hydrolysis of organic phosphate monoesters at alkaline pH. The enzyme is present in practically all tissues of the body, especially at or in the cell membranes, and it occurs at particularly high concentrations in placenta, intestinal epithelium, kidney tubules, osteoblasts and liver.

The form present in the sera of normal adults originates mainly in the liver and bone.

Elevated serum ALP is found in patients with bone disease associated with increased osteoblastic activity (Paget's disease, primary and secondary hyperparathyroidism, bone tumors, rickets, osteomalacia, bone fractures) and also in patients with hepatobiliary disease (obstructive jaundice, hepatitis, hepatotoxicity caused by drugs, liver cancer). Physiological changes, such as bone growth and pregnancy, may cause increases in ALP levels[4,5].

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

NOTES

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

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The lateral lines mark the modifications in the current version.

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