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Role of Matrix Metalloproteinase -9 (Mmp -9) Concentration in The Pathogenesis of Preeclampsia

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Abstract

Background: Preeclampsia is a syndrome related to pregnancy and represented the second common maternal and infant death causes. Metalloproteinase is involved in vascular growth of placenta, and it has been shown to have a role in preeclampsia pathogenesis.

Aim of study: The evaluation of metalloproteinase role in prediction of pre-eclampsia in pregnancy.

Patients and methods: This is a case control study conducted at Obstetric & Gynecological Department of Al-Daquq general hospital in Kirkuk during the period from 1st of March to end of November 2017.A sample of 50 preeclamptic pregnant women, 25 preeclamptic pregnant females of gestational age of (< 37 weeks) and 25 preeclamptic pregnant women with gestational age of (more than 37 weeks) were included in the study. A sample of 50 healthy controls, 25 healthy pregnant women with gestational age of (<37 weeks) and 25 healthy pregnant females with gestational age of (more than 37 weeks).

Results: In contrast to preterm controls, there was a highly important correlation between lower mean Metalloproteinase-9 and pregnant preterm women with preeclampsia ($p<0.001$). In contrast to term controls, a highly significant correlation was observed between lower mean Metalloproteinase-9 and term pregnant women with PE ($p<0.001$). In pre-eclampsia prediction, Metalloproteinase-9 levels of 26.2 for pre-term and 34.4 for term were cut off.

Conclusions: The matrix metalloproteinase 9 is a nonspecific predictor of preeclampsia for term and preterm pregnant women but with higher accuracy for preterm pregnant women.

Introduction

Preeclampsia

Has been described as a pregnancy-specific syndrome which could affect virtually every organ system. [1] It's an idiopathic disorder of pregnancy Characterized by protein uric hypertension, 5 to 10 percent of all pregnancies are complicated by [2]. The presence of proteinuria remains an important feature of diagnosis. Proteinuria is, thus, an objective marker that represents the systemic endothelial leak that characterizes the syndrome of preeclampsia. [3] Disorders of hypertension. [4]

Pre-eclampsia is the second leading cause of both direct maternal death and perinatal mortality, resulting in the death of six to nine women per year in France. [5] It is a multisystem condition of unknown etiology characterized by the occurrence of hypertension at or above 140/90 mm Hg with proteinuria after 20 weeks in pre-normotensive and non-proteinuric females [6]. Every year around the world, ten million women experience preeclampsia. Every year, approximately 76,000 pregnant women suffer from preeclampsia and associated hypertensive disorders worldwide. And it is thought that the number of babies who die from these conditions is on the order of 500,000 every year. [7]A woman is seven times more likely to experience preeclampsia in developing countries than a woman in a developed world. 10-25% of these instances will lead to maternal death.

Epidemiology

The occurrence of pre-eclampsia varies with the term used and the population examined, but pre-eclampsia occurs in less than 5% of the average antenatal population. The incidence was as low as 2.2 per cent in some recent prospective studies, also in a primigravid population in which the disease is considered to have the highest prevalence. Non-protein uric PIH incidence is approximately three times greater. Pre-eclampsia is about 15 percent for women with a single risk factor.

Risk factors.

In addition to raising the risk of maternal mortality and morbidity, preeclampsia is directly related to most fetal adverse effects [i.e., intrauterine growth restriction, preterm birth, birthweight, and perinatal death). [8] In addition, recent findings have shown that preeclamptic females and their offspring are at higher risk of cardiovascular and kidney diseases later in life. [9] There are many identified risk factors for the development of preeclampsia, although its pathophysiology is not fully understood, such as:

A- Maternal risk factors

1. Primigravida.
2. Age <20 or >35.
3. Multiparous along with:
    i. Preeclampsia in former pregnancy.
    ii. My last pregnancy before 10 years ago, or more.
    iii. 40 years of age or more.
    iv. 35 or more body mass index.
    v. Preeclampsia family history (mother or sister).
    vi. Booking 90 mmHg or more of diastolic blood pressure.
    vii. Proteinuria bookings (+1) or more in over than one instance or quantified around 0.3 g/24 hours.
4. Multiple gestation.
5. Previously existing medical problems [10].
    i. Previously existing hypertension.
    ii. Previously existing kidney disease.
    iii. Previously existing diabetes.
    iv. “Antiphospholipid antibodies”.
6. Pregnancy associated factors. [11,12]
    i. Chromosomal abnormality.
    ii. H. mole.
    iii. Hydrops fetalis.
    iv. Multi fetal pregnancy.
    v. “Oocyte donation or donor insemination”.
    vi. Structural “congenital abnormality”.

vii. "Urinary tract infection".

7. Ethnicity. [13]

B- Specific paternal factors

1. first-time father
2. formerly fathered a preeclamptic pregnancy with another female [14].

In recent decades, the higher prevalence of these risk factors in developed countries is most likely the cause of an elevated rate of pre-eclampsia [15]. While fetal and placenta delivery is still the only definitive cure for preeclampsia, unraveling the etiological mechanisms of preeclampsia will provide improved treatment approaches and eventually prevention [16].

Hypertension classification of pregnancy

The classification system proposed by the National Working Group on High Blood Pressure Education Program for hypertension in pregnancy: [5]

Hypertension Definition

- ❖ Mild: 140 mmHg of systolic blood pressure.

90 mmHg of diastolic blood pressure.

- ❖ Severe: 180 mmHg Systolic Blood Pressure.

110 mmHg of Diastolic Blood Pressure.

Chronic Hypertension

Hypertension with onset prior to pregnancy or gestation before 20 weeks. Usage of antihypertensive drugs prior to pregnancy Hypertensive persistence beyond 12 weeks postpartum.

Pre-eclampsia

Hypertension, which occurs in a woman with previously normal blood pressure after 20 weeks of gestation. SBP 140 mmHg or DBP 90 mmHg at least 6 hours apart on two occasions + large protein urea.

Proteinuria is characterized as urinary excretion of 0.3 g or more of protein in a 24-hour urine sample. This result typically correlates with a lipstick finding of 1 or greater. Symptoms and/or biochemical and/or hematological dysfunction [11], including one of the following observations:

- Oliguria every 24 hours with less than 500 mL.
- A recurrent maternal headache or vision distortion or papilledema [17].
- "Pulmonary edema" or cyanosis.
- Abdominal pain issue.
- "Impaired liver" function test results.
- "Thrombocytopenia" [18].
- ➢ Note: Edema is not a diagnostic criterion anymore.
- Note: A systolic increase of 30 mm Hg or a diastolic increase of 1 mm Hg is no longer a requirement for diagnosis.

Eclampsia

"New-onset Grand Mal seizures" that cannot be attributed to other causes in a woman with preeclampsia.

Pre-eclampsia / Eclampsia Superimposed

Pre-eclampsia or eclampsia exist in a female with chronic pre-existing hypertension.

- **Gestational Hypertension**

Hypertension observed for the first time after mid-pregnancy identified from pre-eclampsia due to the absence of proteinuria diagnosis during pregnancy only**.**

- **Transient Pregnancy Hypertension**

Postpartum gestational hypertension resolves after 12 weeks.
If a patient with gestational hypertension experiences proteinuria, the diagnosis is pre-eclampsia. The condition is chronic hypertension if gestational hypertension does not improve by 12 weeks postpartum.

Criteria for Pre-eclampsia and Eclampsia Diagnosis: [19]

Pre-eclampsia:

Initiation of a new hypertension episode in pregnancy, distinguished by:
•Persistent "hypertension" (90 mm Hg of diastolic blood pressure).
• "Considerable proteinuria" (> 0.3 g/24 hours).

Eclampsia:

Generalized seizures, usually in addition to the pre-eclampsia criterion [20]. As the clinical symptoms of pre-eclampsia can be heterogeneous, it may not be easy to diagnose preeclampsia. Preeclampsia can be asymptomatic without extreme features. By regular prenatal screening, several cases are detected. women with serious preeclampsia show end-organ symptoms and can complain about the following:

Early symptoms

1. High blood pressure.
2. proteinuria.
3. Edema (which unexpectedly happens and appears to be more intense).

Late symptoms:

The following symptoms can develop later on:

1. Blurring in sight, seeing blinking lights at times.
2. Headaches, usually serious.
3. "Malaise".
4. Breath Shortness.
5. Ache on the right side just under the ribs.
6. Sudden gain in weight (because of fluid retention).
7. Vomit.
8. Reduction of urine levels.
9. Decrease in blood platelet count.
10. Impaired test for liver function.

Development restriction due to reduced blood flow to the placenta is the key symptom of preeclampsia in the fetus.

Pathophysiology

The etiological agent in charge of preeclampsia production remains unclear. Vasospasm, hemoconcentration, and ischemic changes in the placenta, kidney, liver, and brain define the condition. These anomalies are typically seen in women with extreme preeclampsia, including placental origin, immunological origin, and genetic predisposition hypotheses of causative mechanisms, among others, a great deal of study is devoted to solving the etiology of preeclampsia. Without a definitive etiology, it remains difficult to predict patients at risk for the development of preeclampsia and to influence treatment for this morbid condition.
Pre-eclampsia is a severe illness in pregnant women and is correlated in developed countries with

high maternal morbidity and fatality. Family aggregation of pre-eclampsia was documented in a previous study, which indicates that genetic factors contribute to the pathogenesis of this disease. 25 to 55 percent of the risk of developing pre-eclampsia is attributed to hereditary factors. [21] Pre-eclampsia pathogenesis originates in the placenta. In the absence of fetal tissue [molar pregnancy), the disease will occur, and manifestations of the disease can only resolve after placenta delivery. [22] Any satisfactory hypothesis concerning the etiology and pathogenesis of preeclampsia must take account of the finding that, in women with the following features, gestational hypertensive disorders are more likely to develop: [23]

•They are first introduced to chorionic villi.

•As with twins and hydatidiform mole, they are exposed to a superabundance of chorionic villi.

•They have pre-existing endothelial cell activation disorders or inflammation, such as diabetes or renal or cardiovascular diseases.

• They are predisposed genetically to the development of hypertension in pregnancy.

There are at least two major subtypes distinguished by whether or not endovascular trophoblastic invasion remodeling of uterine spiral arterioles is defective. "This idea has given rise to the pre-eclampsia etiopathogenesis theory of "two-stage disorder. Step 1 is triggered by defective endovascular trophoblastic remodeling that causes the clinical stage 2 syndrome downstream. Importantly, by pre-existing maternal disorders that are expressed by endothelial cell activation or inflammation, stage 2 is prone to alteration. These conditions include cardiovascular or kidney disease, diabetes, obesity, immunological disorders or genetic factors [24]. In turn, these endothelial defects cause hypertension by impairing the function of the kidneys and increasing the overall resistance of the periphery.

The understanding that immunological dysfunction is a significant factor in the pathogenesis of preeclampsia is now increasing. There is a potential role of immune maladaptation in the pathophysiology of preeclampsia. Early in pregnancy, extra villous trophoblasts express reduced levels of immunosuppressive non-classic HLA G in women intended for pre-eclamptic use. [25] Although it is well known that pregnancy alone induces an increased maternal inflammatory response, there is a marked increase in the secretion of inflammatory cytokines in women with preeclampsia. Recent research has shown that the development of tumor necrosis factor-alpha (TNF-alpha) and other inflammatory cytokines is primed by circulating micro particles of syncytiotrophoblast that are elevated in preeclamptic women. [26] By the discovery of an autoantibody agonistic circulating angiotensin II type I receptor (AT1-AA).

In the bloodstream of preeclamptic patients, AT1-AA was identified, and its epitope was eventually mapped to the second extracellular loop of the AT1 receptor. Placental ischemia in pregnant women in response. [27] It is clear that placental vasculogenesis occurs 21 days after conception. Soluble fms-like tyrosine kinase-1 (sFlt-1) is a spliced version of the longer VEGFR-1 cell surface receptor in which post-transcriptional excision of the cytoplasm and trans membrane domains is performed. This molecule, which is formed in its soluble form (sFlt-1) by placental trophoblasts, acts as an antagonist of the proangiogenic proteins VEGF and PlGF by sequestering free protein into the plasma, rendering it inaccessible for receptor binding.[28]

In addition, elevated levels of sFlt-1 were found in preeclamptic women in both placenta and plasma relative to women with a normal pregnancy. [29] Third-trimester elevation of sFlt-1 levels and reduced concentrations of PlGF are correlated with development of preeclampsia after 25 weeks. [30] Matrix metalloproteinase is among the factors that may promote trophoblast invasion (MMPs). [31] MMPs are well-established tissue remodeling and angiogenesis mediators, some of whom exhibit modified expression in PE patient placentas. The extracellular matrix (ECM) is attacked by MMPs and is involved in normal physiology and in various pathologies. MMPs are secreted by extracellular proteinases as inactive proenzymes, which are activated when broken. [32] At the human fetomaternal interface, specifically uterine natural killer cells, decidual cells and trophoblasts, a surprisingly wide range of MMPs and tissue inhibitors of MMPs are expressed [33].

Results of in vitro experiments using a broad-spectrum pharmacological MMP inhibitor affirm their importance in mediating placental growth [34]. MMP9 (92-kDa gelatinase B or collagenase type IV) is a primary ECM remodeling effector that degrades collagen types IV, V, and IX, denatured collagen (gelatin), and elastin. [35] Active MMP9 is highly expressed by human trophoblasts at the embryo implantation site and is implicated in their invasive actions. [36] Many lines of evidence point to the function of MMP9 in PE, as cytotrophoblasts in PE produce less inhibition of MMP9 and MMP9, or in vitro invasion of cytotrophoblasts by gene silencing blocks. [37] MMP9 is also consistently deficient in the plasma of PE patients, and the MMP9 variant has recently been shown to be a useful biomarker of susceptibility to and early onset of serious PE.

Role of MMPs in the pathogenesis of preeclampsia

Trophoblasts are essential human placenta precursor cells that play critical roles in promoting healthy gestation, including embryo implantation, hormone development, fetal immune protection, and vascularization of the placenta. Cytotrophoblast cells invade the uterine tissue and migrate through the maternal spiral arteries against the bloodstream in the first trimester of normal pregnancy, where they are differentiated into endothelial phenotype cells. [38] Trophoblastic invasion of maternal vessels results in the remodeling of the extracellular matrix, which gives rise to high distensibility of the uteroplacental vessel to handle increased blood flow. [39] Reactive oxygen species and tumor necrosis factor-alpha have been involved in pre-eclampsia pathogenesis. [40] Extracellular matrix proteins, especially matrix metalloproteinase proteins, tend to induce vascular expression (MMPs).

MMPs are involved in short-term biological processes, including vascular reactivity control and leukocyte activation, beyond their matrix remodeling properties. [41] Recently, the role of MMPs in pre-eclampsia pathogenesis has aroused interest. MMPs are enzymes dependent on zinc and calcium that play an important role in both physiological and pathological mechanisms. [42] MMPs are involved in the pathogenesis of angiogenesis and vascular remodeling by degrading pre-eclampsia-related extracellular matrix proteins.[43] Evidence also indicates that decreased MMP activity results in weak trophoblastic invasion of the maternal spira [44]

Tissue metalloproteinase inhibitors (TIMPs) are endogenous inhibitors that bind MMPs, and their expression is controlled during development and tissue remodeling. However, trophoblastic invasion is decreased in preeclampsia, leading to incomplete modification of maternal spiral arteries and thus decreases in placental perfusion. In particular, MMP-2 and MMP-9 are involved in the remodeling of the placental and uterine arteries, and abnormal expression of these MMPs has been documented in hypertensive pregnancy disorders. Matrix metalloproteinase (MMPs) activity targets extracellular matrix components during development and morphogenesis [45]. Indeed, there is now evidence that MMPs may have an effect on vascular function and play a role in pre-eclampsia and other cardiovascular diseases. [46] Under normal circumstances, MMP activity is regulated at the level of transcription, activation of latent forms, and inhibition by tissue metalloproteinase inhibitor (TIMP) MMP-9 activity is regulated at different levels, including activation of latent forms of MMP-9, and is also regulated at the transcriptional level by interaction with TIMPs, in particular TIMP-1. Again, it has also been shown that genetic polymorphisms in the MMP-9 gene influence MMP-9 transcription and susceptibility to disease.[47]

These previous studies indicate that vasoconstrictors are apparently produced by imbalanced MMP activity and vasodilators are degraded, promoting vasoconstriction and hypertension. Moreover, the release of the TNF-alpha proform from its membrane-bound site is an MMP-dependent process. [48] In comparison, TNF-al stimulates the proteolytic activity of MMPs indirectly, especially during the implantation phase [49], indicating that abnormal MMPs and inflammatory mediators can interact and correspond to the characteristics of this syndrome.

In addition, oxidative stress and decreased bioavailability of nitric oxide may result in endothelial dysfunction in preeclampsia. Indeed, increased concentrations of reactive oxygen species including superoxide may increase vascular peroxynitrite concentrations, a powerful oxidizing agent that

contributes to the pathogenesis of many cardiovascular species, including preeclampsia. [50] This agent can activate MMPs directly.
By proteolysis of cell surface receptors such as VEGFR-2 and β (2)-adrenergic receptors, activated MMPs can also lead to cardiovascular dysfunction in preeclampsia. [51] These ideas, however, continue to be illustrated in pre-eclampsia. In women who experience preeclampsia before they become symptomatic, several circulating endothelial cell injury markers have been shown to be elevated; these include endothelins, cellular fibronectin, plasminogen activator inhibitar-1. [52] Recent data indicate that circulating factors that interfere with the action of vascular endothelial growth factor (VEGF) and placental growth factor (PIGF) play a major role in the maternal manifestation of this condition, as both (VEGF) and (PIGF) promote placental essential angiogenesis, both of which are secreted by the placenta in women who are critical for placentation.[53]

Complications of preeclampsia

1. 16 percent of maternal deaths have been reported to be attributed to hypertensive disorders in developing countries.
2. Hepatic [54].
   - HELLP syndrome: HELLP syndrome, characterized by haemolysis, elevated liver enzymes and reduced platelet count, will occur in 12 percent of females with serious pre-eclampsia.
   - Intrahepatic hemorrhage and sub capsular hematoma can result from liver ischemia.
3. Renal [55] 1-2 percent of PE females are problematic.
   - Acute tubular necrosis caused by preeclampsia is rarely seen.
   - Invariably, renal failure is attributed to coexisting hemorrhage with hypovolemia and hypotension. Typically, this is due to serious obstetric hemorrhage for which sufficient replacement of blood is not provided.
4. Neurological:[56]
   - Headache and scotomata are believed to originate from the occipital lobes that have a predilection for cerebrovascular hyper perfusion.
   - Convulsions are a second possible manifestation and eclampsia is diagnosed.
   - Blindness.
   - Generalized cerebral edemamay occurs and is normally manifested by changes in mental state that range from coma to confusion.
5. Placenta abruption: Abruption is more frequent, developing in about 1 in 20 of these early onset cases of PE, and about 7 percent of all eclampsia women may have premature placenta separation.
6. Overall, the chance of recurrence is around 16 percent, but if delivered prior to 28 weeks of gestation due to hypertensive disease, this rises to 55 percent [57].
7. Fetal complications [58]: PE is the major cause of "perinatal morbidity and mortality" Intrauterine development restrictions affect up to one-third of infants born from pre-eclamptic pregnancies. The most common cause of iatrogenic prematurity is PE, which accounts for 15 percent of all premature births and about one in five (<1500 g) extremely low birth weight babies.

Management of pre-eclampsia

Diagnosis and screening

Pre-eclampsia screening usually involves examining clinical risk factors such as age, body mass index (BMI) and family history, paired with a 20-week ultrasound scan. An international cohort project, however, concluded that the predictive strength of clinical risk factors was moderate. [59] Many maternal serum markers have recently been tested as novel candidates for detecting pre-eclampsia. Placental growth factor (PIGF), plasma protein A associated with pregnancy (PAPP-A)

[60, 61], Placental protein-13 first trimester [62] and soluble fms-like tyrosine kinase-1 (sFlt-1) [63] are some of the possible biomarkers for pre-eclampsia development detection. A systematic review [64] on the accuracy of PIGF and sFLT-1 (among other studies) concluded, however, that the accuracy of the test was too low to accurately predict pre-eclampsia in clinical practice, although the tests could be useful if integrated into multivariable prediction models. Combined clinical factors and measurements of previously identified pre-eclampsia risk biomarkers in patients recruited for the Low-Risk Nulliparous Female Screening for Pregnancy Endpoints (SCOPE) Study; Again, only a modest prediction of pre-eclampsia was given by integrating multiple biomarkers and clinical and ultrasound results. [65] Therapeutic options for prevention are relatively limited once patients have been identified as at high risk of developing pre-eclampsia.

Treatment

Low-dose aspirin [60-80 mg) is recommended daily before 20 weeks of gestational age by the American College of Obstetricians and Gynecologists (ACOG) and the World Health Organization (WHO). [66] In addition, calcium [1.5–2.0 g per day) is recommended by the WHO, especially in areas where dietary calcium intake is poor [67]. The consistency of the evidence that underlies these guidelines, however, is only moderate. Vitamin C and E supplementation, restriction of dietary salt consumption, and bed rest are not specifically prescribed. The treatment of pre-eclampsia or eclampsia patients consists mainly of increased management, magnesium sulphate for the prevention of eclampsia and seizures, and, at a certain stage, induction of labor [66, 67]. Labor induction naturally requires hospital admission, and, in some cases, increased management can also require hospital supervision.

Patients & Methods

Study design & setting.

This case-control study was conducted at Obstetric & Gynecological Department of Al-Daquq general hospital, Kirkuk through the period from 1st of March to end of November 2017.

Study population.

In this study 100 pregnant women 50 of them with symptoms and signs of pre-eclampsia (PE) 25 cases with gestational age <37 weeks, and 25 cases with gestational age >37 weeks. Second group include 50 cases of pregnant women with normal blood pressure, 25 of them with gestational age >37, and 25 cases of gestational age <37 weeks. Are admitted to the Obstetric & Gynecological Department of Al-Yarmouk Teaching hospital were selected in this prospective study population.

Patient selection:

After taking detail obstetrical and medical history (diabetes, renal disease…etc.), examination done for them which include general and obstetrical examination, in this study, pre-term and term pregnant patients were included.

In general examination, their height in meter and weight in(kg) were calculated. The BMI was determined using the formula (Wt/Ht)2, then their blood pressure were determined using mercury sphygmomanometer (Korotokoff 1-5 is used to describe systolic and diastolic BP) , 50 of them with normal blood pressure without any history of medical condition and 50 of them with severe PE defined using the criteria of severe PE, which include (elevated blood pressure, headache , blurring of vision ,nausea, vomiting, epigastric pain, edema, protein urea.

When their blood samples were taken for serum MMP-9 after oral consent and affordability of investigation, these samples were left to stand at room temperature for at least 30 minutes to let the blood to clot, The serum MMP-9 was tested using ELIZA sandwich kits with the assay range from zero to 100 ng/dl and then centrifuged for 5 minutes and then frozen at-20ºc and kept there without thawing until the day of testing.

Also, urine samples were collected using mid-stream urine or catheter specimens for protein urine dipsticks.
All these results were documented by predesigned form after that statistical analysis were performed.

Inclusion criteria

1. Pre-term pregnancy with PE.
2. Term pregnancy with PE.
3. Pre –term with normal blood pressure
4. Term with normal blood pressure

Exclusion criteria

1. “Chronic hypertension”.
2. “Diabetes mellitus”.
3. Heart diseases.
4. Kidney diseases.
5. Twin pregnancy.
6. Congenital anomalies.
7. “HELLP syndrome (haemolysis, elevated liver enzyme low platelet)”.

Study sampling groups:

A sample of 50 PE pregnant females 25 PE pregnant females with gestational age of (<37) weeks and 25 PE pregnant females with gestational age of more than 37 weeks were included in the study. A sample of 50 healthy pregnant females presented to Clinic of Obstetric and Gynecological Consultancy in Al-Yarmuk Teaching hospital was selected as controls 25 healthy pregnant females with gestational age of (< 37) weeks and 25 healthy pregnant females with gestational age of more than 37 weeks).

Data collection

The full medical history of each woman was taken including the previous pregnancy, past obstetric, past surgical, social, and drug history, including demographic information (name, age, and job).
By completing the prepared questionnaire developed by the researcher and supervisor, the information was collected by the researcher. The questionnaire included the following information:

1. Age of pregnant women.
2. Past obstetrical history: Gravidity, parity and abortion history.
3. Gestational age.
4. Body mass index.
5. Chief complaints.
6. Past medical history.
7. Past surgical history.

Examination

1. Blood pressure.
2. Fundal height.

3.

Investigations

1. Albumin in urine.
2. Liver and renal function test.
3. Metalloproteinase-9 (MMP-9) level.

Follow up.

1. Delivery type, vaginal delivery, or cesarean section.
2. Outcome of pregnancy: Fetal status, gender, and weight.

The supervisor completed the diagnosis of PE based on signs and symptoms such as "high blood pressure, albumin in the urine, and related symptoms such as headache, blurred vision, nausea, vomiting, and epigastric pain". A blood sample of 5 ml of was obtained from each of the selected women and sent to a private MMP-9 laboratory.

Ethical considerations:

- Ethical consent has been obtained from Iraqi Obstetrics & Gynecology Scientific Committee Board.
- Agreement obtained from the administration of Al-Yarmuk Teaching Hospital.
- An oral informed consent was taken from pregnant women.

Statistical analysis

Serial identification number was assigned to each pregnant woman. The data were analyzed by" the application of version 23 of Microsoft excel software and Statistical Package for Social Sciences (SPSS)". The results of the study were organized into variables of scale (means & standard deviation) and categorical variables.

For categorical data comparison the Chi square test was used "(Fishers exact test applied when expected variable was less than 20 percent of total)". To compare two means, the independent sample t-test was used. To forecast the required cutoff values and to detect the outcomes of these values for validity the ROC curve was used. The significance level (p value) was set as ≤ 0.05.

Results

This research included 100 pregnant women in total. according to their gestational age group, 50of healthy pregnant women (controls), and 50 cases included pregnant women with pre-eclampsia (PE).1st group of GA. More than37 weeks subdivided into two groups, 25 preeclamptic patients and 25 cases healthy pregnant women (control). 2nd group GA. (less than 37 weeks), also subdivided into two groups 25 cases with PE and 25 cases control.

No substantial difference in age between preterm PE and preterm control of pregnant women (p=0.3) was observed in the demographic distribution shown in this age group-related table. There was no substantial difference in age between pregnant women with term PE and term control (p=0.3). These results have all been shown in Table 1.

Table 1. Age distribution according to PE and controls for preterm and term pregnant women.

Variable	Control		PE		P
	No.	%	No.	%	
Preterm					
Age					0.3* *Not significant*
<20 years	1	4.0	4	16.0	
20-29 years	9	36.0	9	36.0	
30-39 years	15	60.0	11	44.0	
≥40 years	0	-	1	4.0	
Mean±SD (years)	28.4±4.1		27.9±7.7		0.7**
Age					0.3* *Not significant*
<20 years	7	28.0	6	24.0	
20-29 years	10	40.0	14	56.0	
30-39 years	8	32.0	4	16.0	
≥40 years	0	-	1	4.0	
Mean±SD (years)	25.6±7.3		25.3±7.1		0.8**

**Fishers exact test, **t-test.*

As shown in table 2, there was a highly significant association between preterm pregnant women with low gravidity history and PE (p=0.02). The parity history of preterm pregnant women with PE was considerably lower than the controls (p=0.02). Similarly, the gravidity and parity of term patients with PE were undoubtedly less than that of control women (p=0.001). There was no significant variation between pregnant women with PE and controls regarding abortion history at preterm and term.

As displayed on table 3 and figure 1, no important variation was observed between preterm pregnant women with PE and preterm controls regarding gestational age by LMP (p=0.2). The mean gestational age by late ultrasound of preterm pregnant women with PE was considerably lower than gestational age of preterm controls (p<0.001). For term women, there was no important variation between pregnant women with PE and controls regarding gestational age by LMP (p=0.1). The mean gestational age by late ultrasound of term pregnant women with PE was substantially lower than gestational age of term controls (p<0.001).

Mean BMI of preterm pregnant women with PE was considerably higher than preterm controls (p=0.008) and mean BMI of term patients with PE was considerably higher than term controls (p<0.001). Table 4 and Figure 2 show all these results.

Table 2: Obstetrical characteristics distribution according to PE and controls for preterm and term pregnant women.

Variable	Control		PE		P
	No.	%	No.	%	
Preterm					
Gravidity					**0.02*** *Significant*
Primigravida	4	16.0	13	52.0	
2-4	17	68.0	10	40.0	
>4	4	16.0	2	8.0	
Mean±SD	3±1.4		1.9±1.3		**0.006****
Parity					**0.02*** *Significant*
Nulliparous	7	28.0	15	60.0	
1-3	18	72.0	8	32.0	
>3	0	-	2	8.0	
Mean±SD	1.7±1.4		0.8±0.6		**0.01****
Abortion					0.3*** *Not significant*
No	18	72.0	21	84.0	
Yes	7	28.0	4	16.0	
Term					
Gravidity					**0.001*** *Significant*
Primigravida	14	56.0	12	48.0	
2-4	3	12.0	13	52.0	
>4	8	32.0	0	-	
Mean±SD	2.8±2.3		1.9±1		0.08**
Parity					**0.001*** *Significant*
Nulliparous	15	60.0	14	56.0	
1-3	2	8.0	11	44.0	
>3	8	32.0	0	-	
Mean±SD	1.7±1.2		0.8±0.6		0.07**
Abortion					0.6* *Not significant*
No	22	88.0	23	92.0	
Yes	3	12.0	2	8.0	

Fishers exact test, **t-test, * Chi-square test.*

Table 3: Distribution of gestational age according to PE and controls for preterm and term pregnant women.

Variable	Control		PE		P
	No.	%	No.	%	
Preterm					
Gestational ageby LMP or early US					0.2***** *Not significant*
Mean±SD (weeks)	32.1±2.4		32.7±1.2		
Gestational ageby late US					**<0.001*** *Significant*
Mean±SD (weeks)	31.9±1.6		28.6±2.2		
Term					
Gestational ageby LMP or early US					0.1* *Not significant*
Mean±SD (weeks)	38.3±0.9		38.6±0.6		
Gestational ageby late US					**<0.001**** *Significant*
Mean±SD (weeks)	37.7±0.6		35.6±1		

Fishers exact test, **t-test, * Chi-square test.*

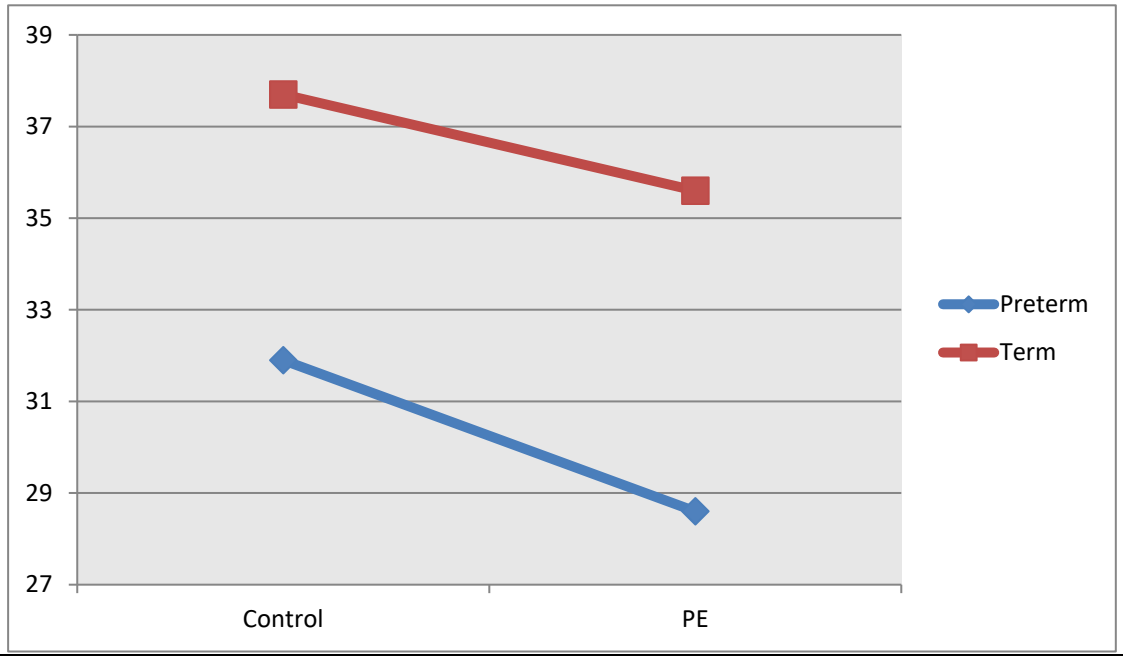

Figure 1: Distribution of GA by late US according to PE and controls for preterm and term pregnant women.

Table 4: Distribution of BMI according to PE and controls for preterm and term pregnant women.

Variable	Control		PE		P
	No.	%	No.	%	
Preterm					
BMI					**<0.001*** *Significant*
Overweight	17	68.0	0	-	
Obese	8	32.0	25	100.0	
Mean±SD (weeks)	30.4±2.7		32.6±23.7		**0.008****
Term					
BMI					**<0.001*** *Significant*
Overweight	14	56.0	0	-	
Obese	11	44.0	25	100.0	
Mean±SD (weeks)	29.6±1.5		34.6±3.1		**<0.001****

**Chi-square test, **t-test.*

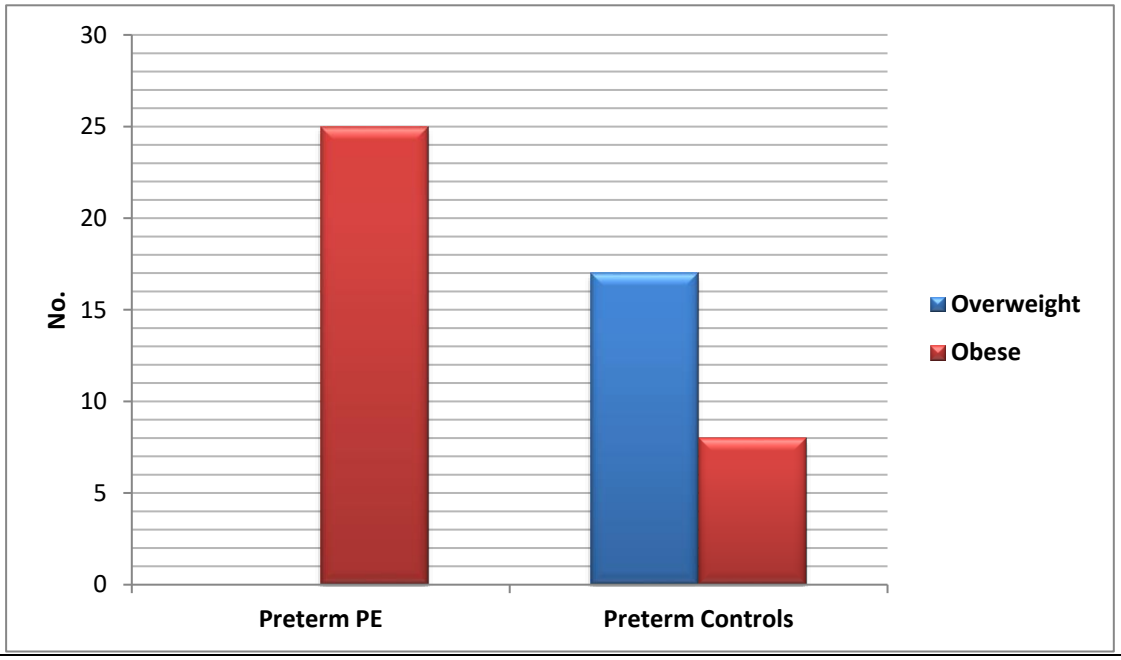

Figure 2: Distribution of BMI according to preterm PE and preterm controls.

As shown in table 5, no major differences were observed between preterm pregnant patients with PE and preterm controls regarding past medical history (p=0.2). The past surgical history was significantly more positive among preterm controls than preterm pregnant women with PE (p=0.04). There was an incredibly valuable relationship between preterm pregnant women with PE and increased each of blood pressure and protein in urine (p<0.001). An important association was noticed between preterm pregnant women with PE and decreased fundal height (p<0.001). For term women, there was a highly significant association between positive past medical history and patients with PE (p=0.002), in same way, positive past surgical history was significantly more among term pregnant women with PE (p=0.01). The positive protein in urine and blood pressure were considerably higher among term patients with PE (p<0.001). Mean fundal height was significantly lower among term pregnant women with PE (p<0.001).

As displayed in figure 3 table 6, there was a highly noticeable association between preterm pregnant women with PE and cesarean section, while preterm controls were mainly managed with conservative ways (p<0.001). The significant mode of delivery for term pregnant women with PE was cesarean section (p=0.005). The fetal outcomes for both term pregnant women with PE and term controls were alive. There was a significant association between term pregnant women with PE and fetal female gender (p=0.04). The mean fetal weight among pregnant women with PE was considerably lower (p=0.003).

Table 5: Distribution of clinical history and findings according to PE and controls for preterm and term pregnant women.

Variable	Control		PE		P
	No.	%	No.	%	
Preterm					
Past medical history (Diabetes, hypertension)					0.2* *Not significant*
Positive	3	12.0	1	4.0	
Negative	22	88.0	24	96.0	
Previous history of C/S					**0.04**** *Significant*
Positive	13	52.0	6	24.0	
Negative	12	48.0	19	76.0	
Albumin in urine					**<0.001**** *Significant*
Positive	0	-	25	100.0	
Negative	25	100.0	0	-	
Blood pressure					**<0.001**** *Significant*
Mean±SD (mmHg)	123/75±5.6/2.8		172/114.8±16/8		
Fundal height					**<0.001**** *Significant*
Mean±SD (cm)	31.3±2.9		28±2.3		
Term					
Past medical history					**0.002*** *Significant*
Positive	0	-	8	32.0	
Negative	25	100.0	17	68.0	
Past surgical history					**0.01*** *Significant*
Positive	0	-	5	20.0	
Negative	25	100.0	20	80.0	
Albumin in urine					**<0.001**** *Significant*
Positive	0	-	25	100.0	
Negative	25	100.0	0	-	
Blood pressure					**<0.001**** *Significant*
Mean±SD (mmHg)	118.4/77±7.7/3.2		166.4/110.4±9/6.6		

Fundal height			**<0.001**** *Significant*
Mean±SD (cm)	36±0.00	34.7±1.6	

Fishers exact test, **t-test, * Chi-square test.*

Table 6: Distribution of delivery modes and outcome according to PE and controls for preterm and term pregnant women.

Variable	**Control**		**PE**		**P**
	No.	**%**	**No.**	**%**	
Preterm					
Mode of delivery					**<0.001*** *Significant*
Vaginal	0	-	2	8.0	
CS	3	12.0	23	92.0.0	
Conservative management	17	68.0	0	-	
Conservative management &tocolytics	5	20.0	0	-	
Term					
Mode of delivery					**0.005*** *Significant*
Vaginal	22	88.0	13	52.0	
CS	3	12.0	12	48.0	
Fetal status					-
Alive	25	100.0	25	100.0	
Fetal gender					**0.04***** *Significant*
Male	13	52.0	6	24.0	
Female	12	48.0	19	76.0	
Fetal weight					**0.001***** *Significant*
Low	2	8.0	13	52.0	
Normal	23	92.0	12	48.0	
Mean±SD (gm)	3016.2±932.7		2396±290.4		**0.003****

Fishers exact test, **t-test, * Chi-square test.*

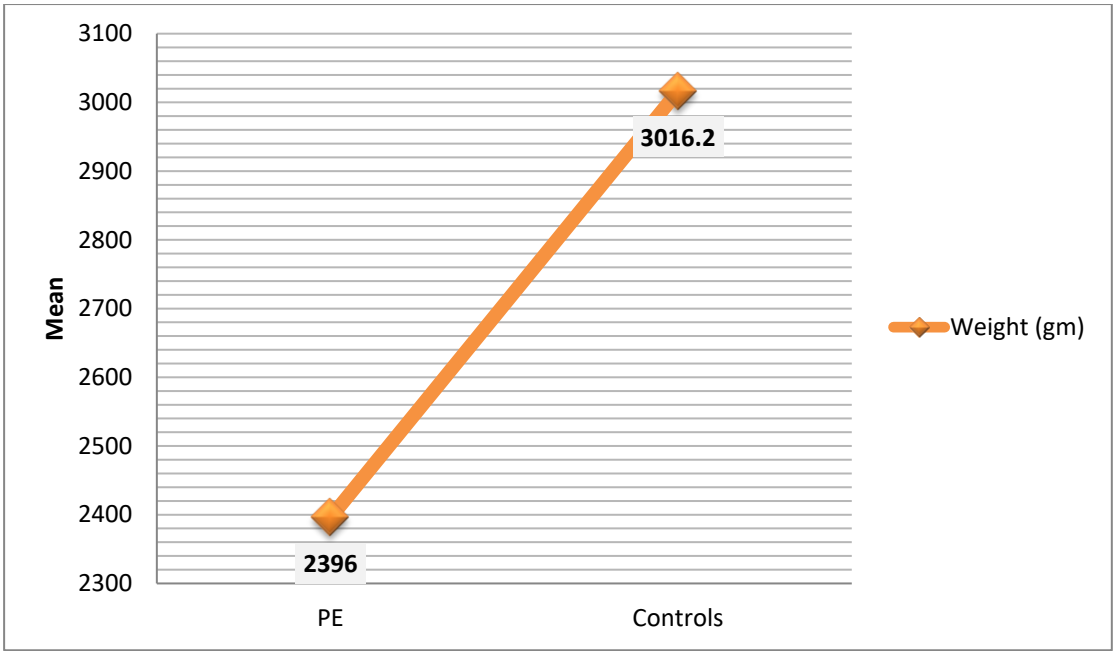

Figure 3: Distribution of fetal weight mean according to term PE and term controls.

As shown in table 7 and figures4 and 5, there was a highly significant association between lowermean of Metalloproteinase-9 and pregnant preterm women with PEin comparison to preterm controls ($p<0.001$). A highly important relationship has been noted between lower mean of Metalloproteinase-9 and term pregnant women with PEas compared to term controls ($p<0.001$).

Table 7 Distribution of Metalloproteinase-9 according to PE and controls for preterm and term pregnant women.

Variable	Control	PE	t-test	P
	Mean±SD	Mean±SD		
Preterm				
Metalloproteinase-9	31.7±3.4	22.5±3	10.1	**<0.001** S
Term				
Metalloproteinase-9	37.7±5.6	31.8±5.9	3.6	**0.001** S

S Significant.

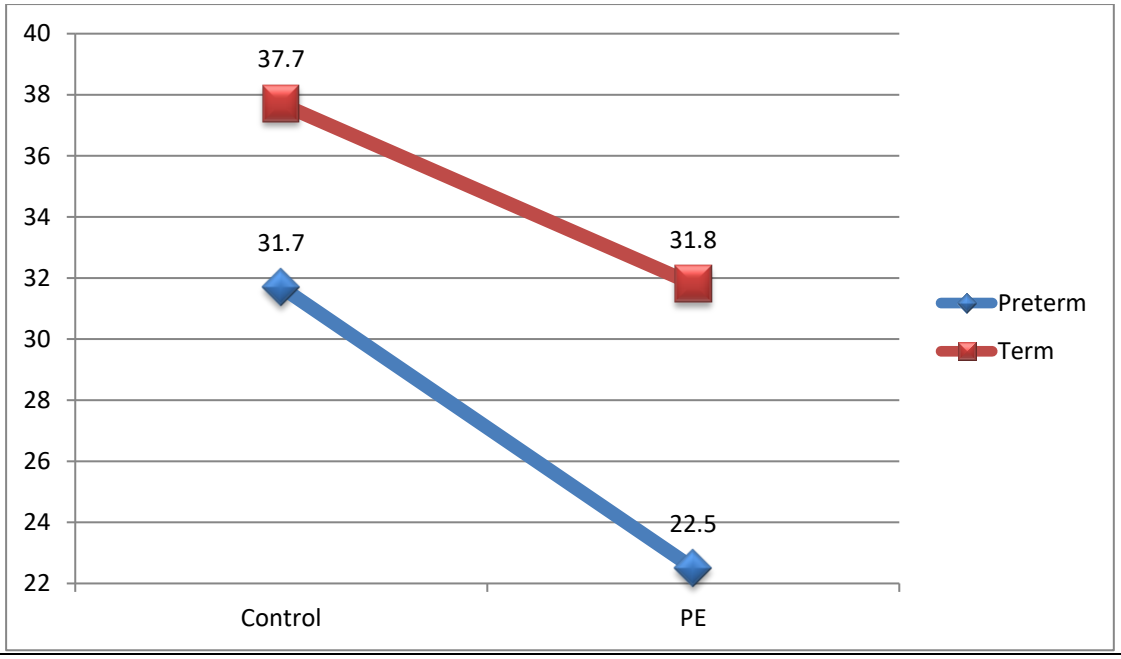

Figure 4: Distribution of MMP-9 according to PE and controls for preterm and term pregnant women.

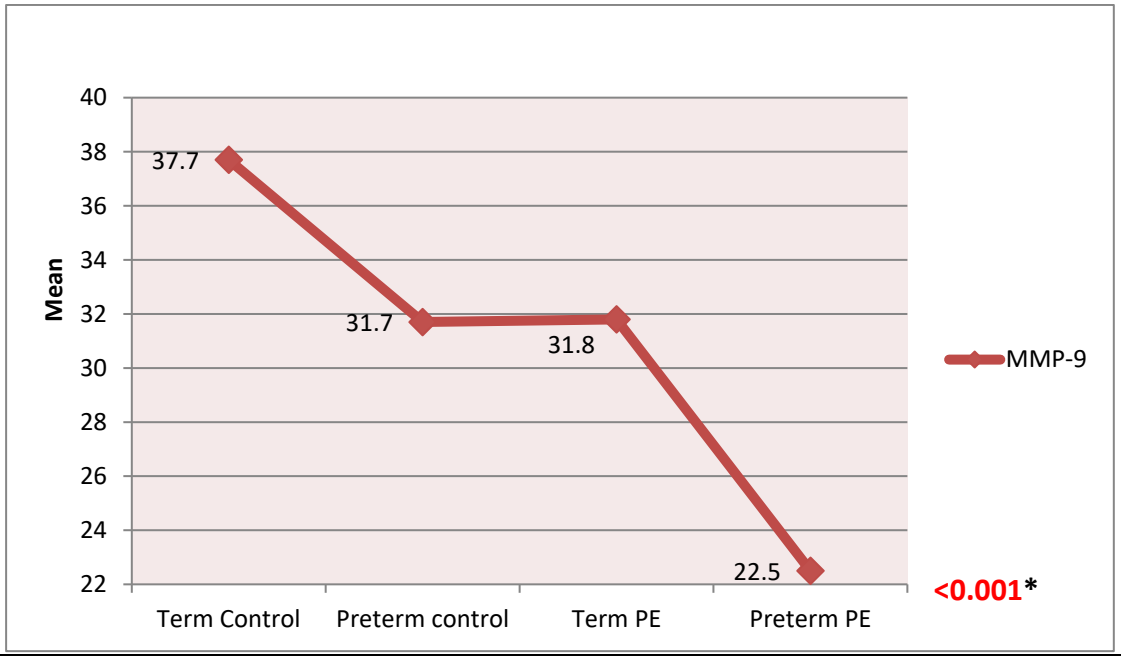

Figure 5: Distribution of MMP-9 mean according to four study groups (*_ANOVA_).

The acceptable cut off points and the corresponding validity tests values forMMP-9 in prediction of PE from healthy pregnant women at preterm were shown in table 9 and figure 6, cutoff MMP-9 level of 26.2 had acceptable validity results (100% sensitivity,96% specificity, 100%PPV, 95.6% NPV and accuracy 97%). MMP-9 levels less and equal to 26.2 was highly predictive of PE in preterm pregnancy.

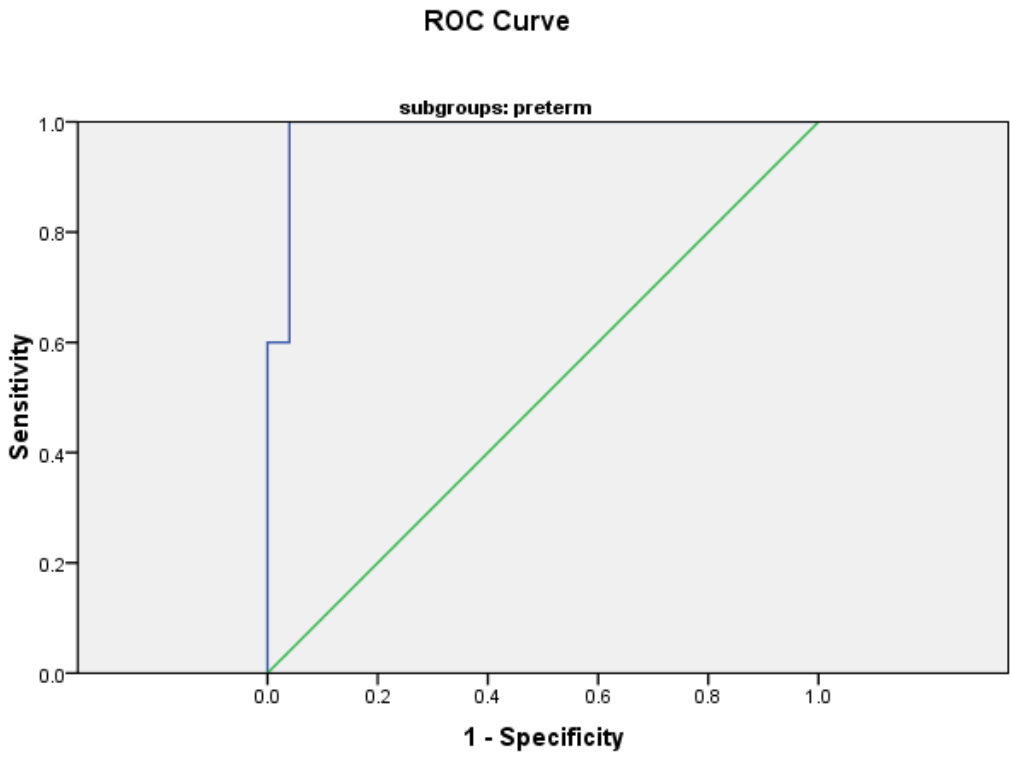

Figure 6: ROC curve for MMP-9 prediction of PE(AUC=0.98).

Table 8: Coordinates of the ROC Curve of MMP-9 regarding PE.

Cutoff point	Sensitivity	Specificity	PPV	NPV	Accuracy
26.2	100%	96%	100%	95.6%	97%

The acceptable cut off points and the corresponding validity tests values for MMP-9 in prediction of PE from healthy pregnant women at term were shown in table 10 and figure 7, cutoff MMP-9 level of 34.4 had acceptable validity results (76% sensitivity, 68% specificity, 69%PPV, 62% NPV and accuracy 70%). MMP-9 levels less and equal to 34.4 was predictive of PE in term pregnancy.

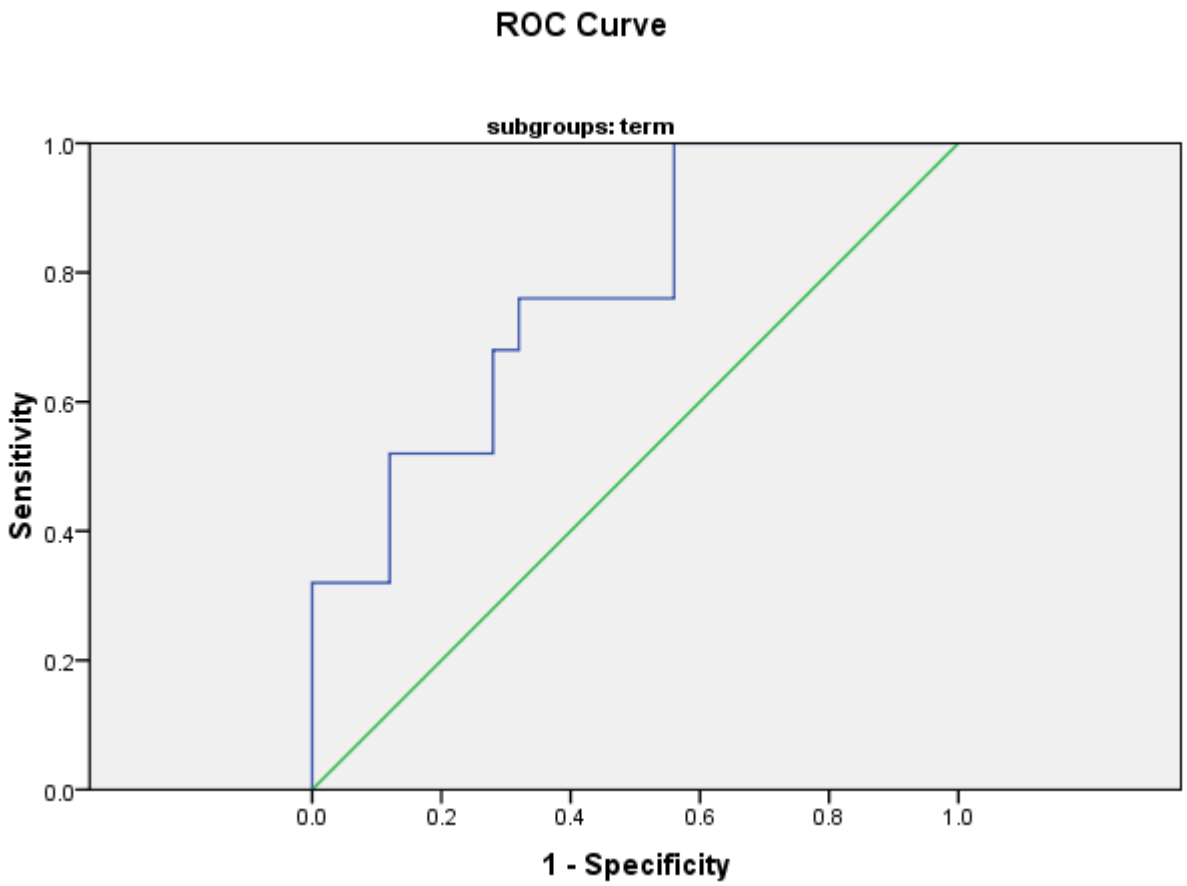

Figure 7: ROC curve for MMP-9 prediction of PE (AUC=0.77).

Table 9: Coordinates of the ROC Curve of MMP-9 regarding PE.

Cutoff point	Sensitivity	Specificity	PPV	NPV	Accuracy
34.4	76%	68%	69%	62%	70%

Discussion

Preeclampsia is a dangerous obstetric condition associated with high maternal morbidity and mortality rates in developing countries in particular [68]. Many diagnostic techniques and biomarkers were developed for early detection of preeclampsia such as metalloproteinase, which had been investigated PE for understanding it's mechanisms and pathogenesis.[69] Current study showed significantly lower levels of metalloproteinase 9 among preterm and term patients with preeclampsia in comparison to healthy patients ($p<0.001$). This finding is consistent with results of Plaks (2013) [70] research in USA and Laskowska (2017) [71] research in Poland they found a significantly decline in MMP-9 level among pregnant women with PE. Consistently, Narumiya et al (2001) in Canada documented low levels of MMP-9 for pregnant women with preeclampsia showed. [72] Many authors detected the relationship between role of metalloproteinase and preeclampsia that is attributed to their effect in remodeling of vessels, angiogenesis and vasodilatation in normal pregnancy. [73] Several literatures found that matrix metalloproteinase-9 level elevated among normal and preeclampsia pregnancy. [74] Coolman et al (2007) [75] reported that higher levels of MMP9 among healthy pregnant women are essential for appropriate development of maternal-fetal

interface. A decreased level of MMP-9 is highly related to angiogenesis impairment and dysfunctional trophoblast invasion that is associated to increased blood vessels resistance and placental dysfunction. [76] Previous study conducted in UK showed low MMP-9 levels among pregnant women with gestational hypertension. [77] The low levels of MMP-9 were also detected among pregnancies complicated with intrauterine growth restriction. [78]

Present study showed significantly lower levels of MMP-9 among preterm pregnant women (PE & controls) than term pregnant women ($p<0.001$). That is similar to results of Sosa (2017) [79] studies in Mexico which noted higher level of metalloproteinase at term normal pregnancies as compared to preterm pregnancies. It was detected that MMP-9 serum levels were increased among pregnant women with preeclampsia and reach peak at first and third trimester of pregnancy. [80] These imbalances in MMP-9 level during pregnancy and between MMP-9 and its inhibitors had bad effect on structure and function of vasculatures among pregnant women with preeclampsia which appeared before the clinical signs of preeclampsia.[79]

Our study revealed that MMP-9 level of 26.2 is significantly predictive for preeclampsia among preterm pregnant women (sensitivity 100%, specificity 96% and accuracy 97%). This result corresponds with Babacan's findings [81] study in Turkey which declared that MMP-9 level of 23.5 is significant predictor of preeclampsia among preterm pregnant women. Another research that was done by Myers et al in UK ((2005) [42] on patents with preeclampsia and normal women at early and late pregnancy found significantly highly MMP-2 activity at early pregnancy for prediction of preeclampsia with no MMP-9 predictive activity.

Our study found that MMP-9 level of 34.4 is significant predictor of preeclampsia in term pregnancy with validity findings lower than that for preterm pregnant women (sensitivity 76%, specificity 68% and accuracy 70%). This data is close to the results Poon (2010) [82] a study in UK reported that MMP-9 level of 53.2 is significant predictor of preeclampsia in late pregnancy but with lower accuracy than MMP-9 prediction of preeclampsia in early pregnancy. A previous study carried out by Laskowska et al (2017) [21] in Poland on 125 patients, 29 preterm preeclampsia, 31 term preeclampsia and 65 healthy pregnant patients (controls) found significantly MMP-9 lower levels among pregnant women with preterm and term preeclampsia than healthy women which may be used as an early preeclampsia diagnostic marker. This Polish study also found that predictive validity results of MMP-9 among preterm pregnant women is better than that for term pregnant women. [22] Previous American study [83] detected a negative association between active MMP-9 expression in healthy pregnancy and gestational age with no important differences of MMP-9 levels regarding preeclampsia. Prochazka et al (2015) documented that MMP-9 level in pregnant women with preeclampsia was irrespective of pregnancy trimester as compared to normal pregnancy. [84]

This study found that the incidence and parity of term and preterm pregnant women with preeclampsia were substantially lower than that of normal stable pregnant women with gravity and parity. This finding is similar to the findings of studies by Saadat et al (2007) [85] in Iran that showed that normal pregnant women had higher levels of gravity and parity than pre-eclampsia pregnant women. A study conducted in Macedonia found that hypertension caused by pregnancy is more common among the females of young primiparas.[86] Preeclampsia is frequently affecting the primigravida women more than multiparous women. Pridjian et al (2002) study in USA referred to multiparty as protective factor of pregnant women from preeclampsia but they stated that multiparty with different partners is preeclampsia risk facto and This may be due to the protective impact of extended exposure to particular antigens [87]

By late ultrasonography, the gestational age of term and preterm patients with preeclampsia was considerably lower than gestation al age of normal pregnant females ($p<0.001$). This result is similar to the findings of Backes et al (2011) [88] study in USA which stated that the outcome of pregnant women with preeclampsia was predominantly immature infants with gestational age 34-36 weeks. In the USA, about two thirds of ten years (1992-2002) raise of preterm births was attributed to incidence of late-preterm deliveries. [89] The reason for this increase is mainly due to obstetrical and pediatric

differences regarding late-preterm births as functionally full term. [90]
The maternal body mass index means of both preterm and term PE pregnancies in our study was significantly higher than BMI of healthy pregnancies ($p<0.001$). These findings are similar to Sohlberg results (2012) [91] study in Sweden which mentioned that short stature and high BMI of women are major risk factors of preeclampsia in all severity types. Consistently, Young et al (2016) [92] study in USA stated that risk of preterm pregnancy preeclampsia is increased with increase BMI of women pre-pregnancy. Present study showed that main chief complaints of preterm pregnant women with PE were headache, vomiting, blurring of vision and eclampsic fits ($p<0.001$). These results are consistent with the findings of the Koike et al [93] research. in Japan. The main chief complaints of term pregnant women with PE in current study were headache, nausea and epigastric pain ($p<0.001$). This is close to reports of Pennington et al (2012) [94] study in USA which found that headache is the most common chief complaint of preeclampsia among term pregnant women.
Term pregnant women with positive previous medical history in present study were significantly at higher risk of preeclampsia than healthy women ($p=0.002$). This is differed with reports of About Al-Hassan et al (2014) [95] study in USA which stated that deteriorated health status of women before pregnancy is regarded as a risk factor for PE development. The blood pressure and albumin in urine for preterm and term preeclampsia patients in our study were substantially higher than that for healthy pregnant women ($p<0.001$). Similarly, previous study conducted in Germany revealed that measurement of blood pressure and albumin in urine must be used as screening parameters of preeclampsia. [96] The fundal height of preterm and term pregnant women with preeclampsia in present study was substantially shorter than that for normal pregnant females ($p<0.001$). This differ from the results of Krishna (2011) [97] research in India mentioned that placental insufficiency among pregnant women with preeclampsia is related to shorter fundal height and intrauterine growth restriction.
Our study showed that preterm and term pregnant women with previous cesarean section history were significantly associated with preeclampsia ($p<0.001$). Cho (2015) [98] in South Korea a study found in a first pregnancy, a cesarean section increases the risk of preeclampsia in subsequent pregnancies. Female fetal gender in this study was significantly associated with preeclampsia of women ($p=0.04$). This is differed from results of Khalil et al (2013) [99] study in Libya which found that outcome of preeclampsia pregnant women was commonly females. In present study, the fetal weight mean of preeclampsia patients was significantly lower than that for normal pregnant women ($p=0.003$). This finding coincides with results of Xiong et al (2002) [100] study in Canada which mentioned that children delivered from term pregnant women with preeclampsia had significantly lower birth weight.

Study limitations.

1. Loss to follow up.
2. Small sample size.
3. Single center study.

Conclusions

- The matrix metalloproteinase 9 is a nonspecific predictor of preeclampsia for term and preterm pregnant women, but with higher accuracy for preterm pregnant women.
- The matrix metalloproteinase 9 level is variable according to gestational of preeclampsia and healthy pregnant women.

Recommendations

- Physicians and Gynecologist could use matrix metalloproteinase 9 as a screening and diagnostic tool for preeclampsia early diagnoses.

- ❖ The matrix metalloproteinase 9examinations could be used for differentiation between term and preterm pregnancy.

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