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Combined Maternal Serum C3 Activation and Uterine Artery Doppler at 14-20 Weeks as Predictors for Pre-Eclampsia in Primigravida

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ABSTRACT

Background*:* Preeclampsia is a multisystem disorder of pregnancy, which complicates 3%-5% of pregnancies in the western world. It is a major cause of maternal morbidity and mortality worldwide. The cardinal clinical features of the condition are hypertension and proteinuria occurring after 20 weeks gestation in women who were not previously known to be hypertensive.

Objective: This study was aimed to assess the efficacy of C3 estimation and measurement of bilateral uterine artery Doppler before 20 weeks of pregnancy for prediction of preeclampsia in primigravida**.**

Methods*:* The study was performed at the Antenatal Care Unit, Obstetrics and Gynecology Department, Sayed Galal Hospital, Al Azhar University on 131 pregnant women at 14-20 gestational weeks during period from July 2017 to December 2017 gestation attending.

Results: On follow up the population of the study 131 pregnant women had completed the study that were classified to 119 (90.8%) with no pre-eclampsia and 12 (9.2%) developed preeclampsia, As regards the Patients characteristics there was no statistical significant difference between the two groups as regard age, height, weight, gestational age, SBP and DPB at enrollment (p-value>0.05). There was a significant difference regarding BMI as (p value < 0.05), with more increasing BMI and decreasing gestational age at delivery in preeclampsia group in comparison with the no preeclampsia group. As regards the C3, there was statistically highly significant difference between the two groups regarding serum C3 level as p value <0.05, with lower levels of C3 serum levels in preeclampsia group. Receiver operator characteristics (ROC) curves were constructed for estimating the association between pre-eclampsia and serum C3 level. A significant association was found with serum C3 level being a significant predictor with lower values in cases with pre-eclampsia than in normal cases [area under the curve (AUC) = 0.935, 95% CI (0.878 to 0.9711.35), best cut off (≤53.1), sensitivity of 83.3%, specificity of 100% positive predictive value (PPV) of 100% and negative predictive value (NPV) of 98.3%.

Conclusion*:* This study demonstrates that lower level of maternal serum C3 in the early second trimester (14-20 weeks gestation) and abnormal increasing in uterine artery indices (PI and RI) are associated with developing pre-eclampsia several months later in pregnancy.

Keywords: Predictors for Pre-eclampsia in Primigravida, Maternal Serum C3 Activation, Uterine Artery Doppler.

INTRODUCTION

Preeclampsia is a multisystem disorder of pregnancy, which complicates 3%-5% of pregnancies in the western world. It is a major cause of maternal morbidity and mortality worldwide. The cardinal clinical features of the condition are hypertension and proteinuria occurring after 20 weeks gestation in women who were not previously known to be hypertensive. Other signs and symptoms include edema and headache, and in severe cases, the condition is associated with seizures (eclampsia), liver, and kidney dysfunction as well as clotting abnormalities, Adult Respiratory Distress Syndrome and fetal growth restriction (FGR) **(1).**

Preeclampsia is one the leading causes of maternal, as well as perinatal morbidity and mortality, even in developed countries. Despite intensive research efforts, the etiology and pathogenesis of preeclampsia are not fully understood. Increasing evidence suggests that an excessive maternal systemic inflammatory response to pregnancy with activation of both the innate and adaptive arms of the immune system is involved in the pathogenesis of the disease **(2).**

The current theory of the pathogenesis of PE as reviewed b y Christopher Redman and Ian Sargent is thought to occur as a 2-stage process with poor placentation in the first half of pregnancy resulting in the maternal response in the second half of pregnancy **(3)**.

PATIENT AND METHODS

Study Type: Longitudinal, prospective study.

Study settings: The study was performed at the Antenatal Care Unit, Obstetrics and Gynecology Department, Sayed Galal Hospital, Al Azhar University on 131 pregnant women at 14-20 gestational weeks during period from July 2017 to December 2017 gestation attending.

Study population: The Study was held on 140 patients.

The study was approved by the Ethics Board of Al-Azhar University.

Sample size justification:

- Sample size at 140 cases, the “p” for the

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changes in both specificity and sensitivity = 0.0357 and 0.0313, respectively (power of the test changes by 8% and 2%, respectively) i.e., changes in both specificity and sensitivity within 140 cases was successfully detected.

- Expected frequency of the disease among unexposed group =6-8%[4].
- The total sample was 140.

Inclusion criteria:

1. primigravida.
2. Singleton pregnancy.
3. Alive fetus.
4. Gestational age between 14-20 weeks.
5. Normotensive women.
6. Absence of congenital anomalies.

Exclusion criteria:

1. Multipara
2. Multiple gestations.
3. Collagen diseases e.g.,: Systemic lupus erythromatosus.
4. Chronic hypertension.
5. Causes of secondary hypertension e.g.,: renal disease.
6. Dead fetus.
7. Diabetes mellitus.

Intervention:

- After approval of the ethical committee, each procedure was explained in detail for each patient.
- All recruited patients were given an informed written consent.
- All patients were subjected to the following:

1-Careful and detailed history:

A) Personal history:

- Name, age, occupation, residence, socioeconomic standard and special habits of medical importance.

B) Obstetric history:

- First day of last menstrual period, estimated gestational age by date and antenatal care.

C) Past history:

- History of diabetes mellitus, hypertensive disorders, cardiac problems, renal diseases, coagulation disorder.

D) Surgical history:

- Previous operations and previous laparotomies.

E) History of the present pregnancy:

- Medical or surgical condition to define high risk factors

2-Examination of patients:

A) General examination:

- Level of consciousness and orientation.
- Maternal body weight, height and calculation of BMI.
- Presence of petichae or ecchymosis of the skin to include presence of coagulation defects or blood disease.
- Vital data (measurement of blood pressure, pulse and temperature).
- Presence of pallor or jaundice.
- The size of the uterus.
- Presence of scar of previous laparotomies.
- Fetal heart rate.

B) Local vaginal examination:

- Vaginal infection.
-Signs of pregnancy.

C) Routine Investigation:

- Hemoglobin.
- RH and blood group.
- R B S.

3) Doppler on Uterine Artery:

- This is a longitudinal, prospective study of women booking for routine antenatal care.
- Primigravida attending for routine second-trimester ultrasound assessment were offered the option to participate in the study, which was approved by the local ethics committee.
- If informed written consent was obtained, transabdominal uterine artery Doppler assessment was undertaken.
- Transabdominal ultrasound was done to assess the uterine artery using MEDISONE X6 ultrasound machine equipped with C 3-7MHZ CONVEX PROBE by the same operators,at the Obstetrics and Gynecology department, Sayed Galal Hospital Al Azhar University.
- All pregnancies were dated by BPD,AC and FL
- The RI (for either side) was defined as abnormal if it was > 0.584 or very abnormal if it was ≥ 0.7, which represented the 95th centile cut-off in a low-risk population.

4) C3 assessment:

Sampling method:

- Use fresh or deep frozen serum samples.
- Blood samples should be collected by venepuncture, allowed to clot naturally and the serum separated as soon as possible to prevent haemolysis.
- The serum may be stored at 2- 8 C for up to 48 hours prior to assay, or for prolonged storage, alliquoted and kept at –20C or below.
- Repeated freezing and thawing should be avoided.

5) Follow up of the patient:

- Traditional patterns of antenatal care advocated around 13 visits with blood

pressure testing at 12, 16, 20, 24, 28, 30, 32, 34, 36 then weekly until delivery.

-For the study testing group:

- Every month till 28 weeks, then every 2 weeks till 36 week then every week till delivery.

- **Initial visit include:**
  1- Clinical evaluation: history, general examination, abdominal and vaginal examination.
  2- Routine antenatal investigation (screening tests) e.g.,: R.H., blood group ,H.B. % , R.B.S .

- **Subsequent visits:**
  1- Ask the patient about any complain.
  2- Examination: blood pressure, Weight, abdominal examination.
  3- Urine for albumin and sugar.
  4- From 32 weeks assessment of the lie, presentation and position of the fetus.
  5- At 36 weeks pelvic assessment may be done.

6) Data Recorded will include:

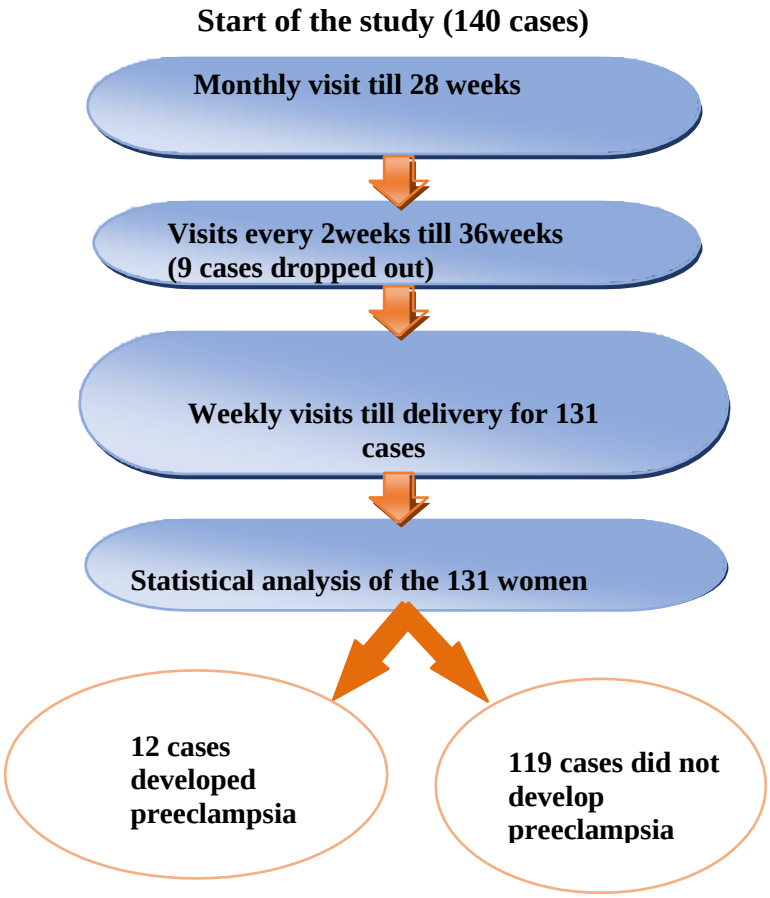
- Maternal age (years).
- Body mass index (kg/m^2).
- Gestational age at delivery (weeks).
- Doppler on uterine artery:
  .Resistant Index (on either side).
  .Persistance or absence of diastolic notch (on either side).
  .Pulsatility index.
- C 3 assessment either it is activated or not (as evidenced by hypocomplementemia).
- Percentage of women that develop preeclampsia during pregnancy.
- Percentage of women that are normotensive at time of delivery.

7) Statistical Analysis and Results:

Statistical analysis was done using IBM© SPSS© Statistics version 21 (IBM© Corp., Armonk, NY, USA) and MedCalc© version 12.5 (MedCalc© Software bvba, Ostend, Belgium). Continuous numerical data were presented as mean and SD and inter-group differences were compared using the unpaired Student t test. Qualitative variables were presented as number and percentage and between-group differences were compared using the Pearson chi square test or Fisher‟s exact test, when appropriate. The DeLong method was used for comparison of the areas under various ROC curves.

A two-sided p-value <0.05 was considered statistically significant and <0.001 was considered highly statistically significant.

RESULTS



In our study 131 pregnant women had completed the study and are classified to 119 (90.8%) with no pre-eclampsia and 12(9.2%) developed preeclampsia that are further classified to 8(6.1%) mild cases and 4(3.1%) severe cases.

Table (1): Classification of the study population according to the development of pre-eclampsia.

	Number	Percent of total
No pre-eclampsia	119	90.8
Pre-eclampsia	12	9.2
-Mild pre-eclampsia	8	6.1
-Severe pre-eclampsia	4	3.1

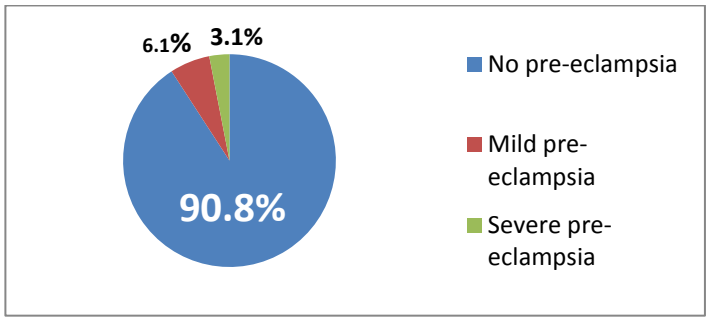


Figure (1): Pie chart showing the rate of development of pre-eclampsia.

There was no statistical significant difference between the two groups as regard Age, Height, Weight and Gestational age at enrollment (p value > 0.05). There was a significant difference regarding BMI as (p value < 0.05), with more increasing BMI and decreasing gestational age at delivery in preeclampsia group in comparison with the no preeclampsia group

Table (2): Patients characteristics:

	All population (n=131)		No Pre-eclampsia		Pre- eclampsia (n=12)		
Variable	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**p-value**
Age (years)	25.1	5.1	24.8	4.9	27.8	6.3	0.058
Weight (kg)	68.0	13.3	67.2	13.4	75.0	10.2	0.054
Height (cm)	161.4	6.5	161.5	6.5	160.3	7.2	0.519
BMI (kg/m^2)	26.1	5.2	25.7	5.0	29.4	5.2	**0.018***
Gestational age at enrollment (weeks)	18.4	1.7	18.4	1.7	18.3	1.8	0.869
Gestational age at delivery	38.2	1.2	38.3	1.1	36.9	2.1	**0.046***

*significant,BMI: body mass index.

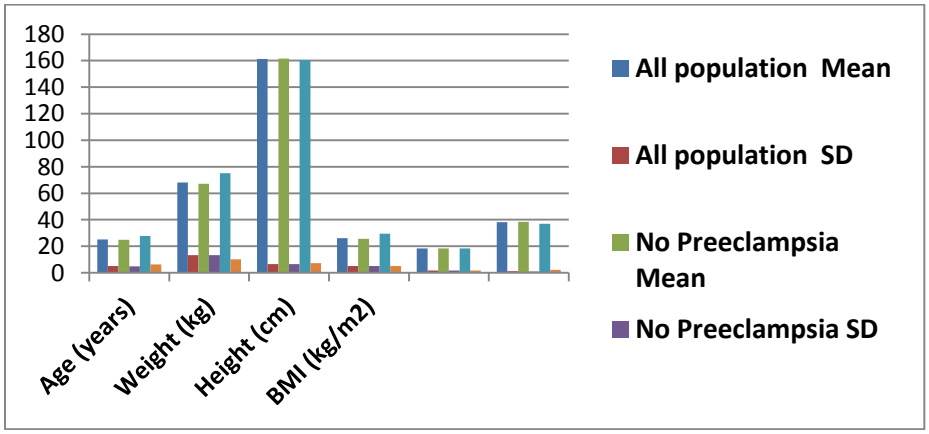


Figure (2): Patient characteristics in patient with or without pre- eclampsia.

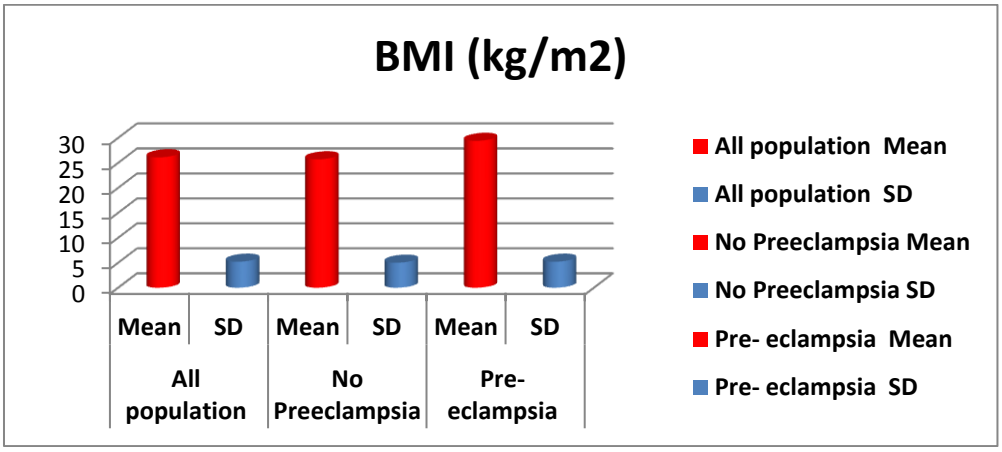


Figure (3): Mean BMI in patients with or without pre- eclampsia. Error bars represent 95% CI.

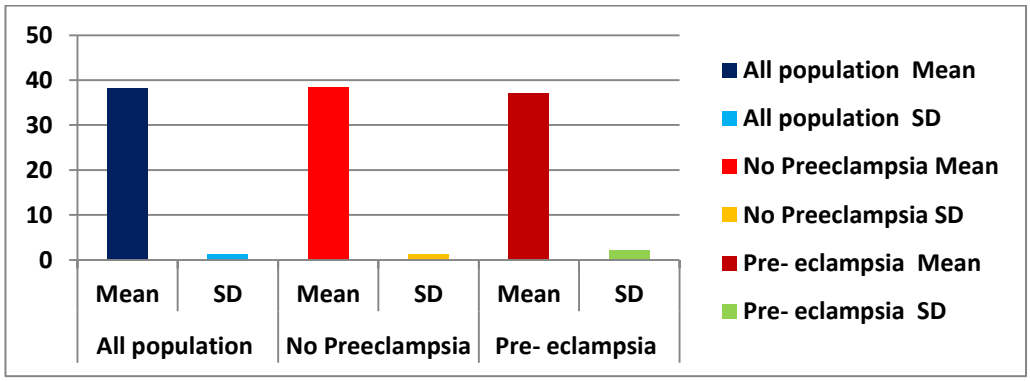


Figure (4): Mean gestational age at delivery in patients with or without pre-eclampsia. Error bars represent 95% CI.

There was no statistical significant difference between the two groups as regards SBP and DPB at the first visit for both groups (p value > 0.05).

Table (3): Blood pressure at first visit:

Variable	All Population (n=131)	No Pre-Eclampsia (n=119)	Pre-Eclampsia (n=12)	p-value
SBP (mmHg)	108.4 (12.0)	108.0	112.5	0.215
DBP (mmHg)	70.1 (9.6)	69.7 (9.3)	73.8 (12.5)	0.170

Data are presented as mean (SD) or number (%).
SBP: systolic blood pressure, DBP: diastolic blood pressure.

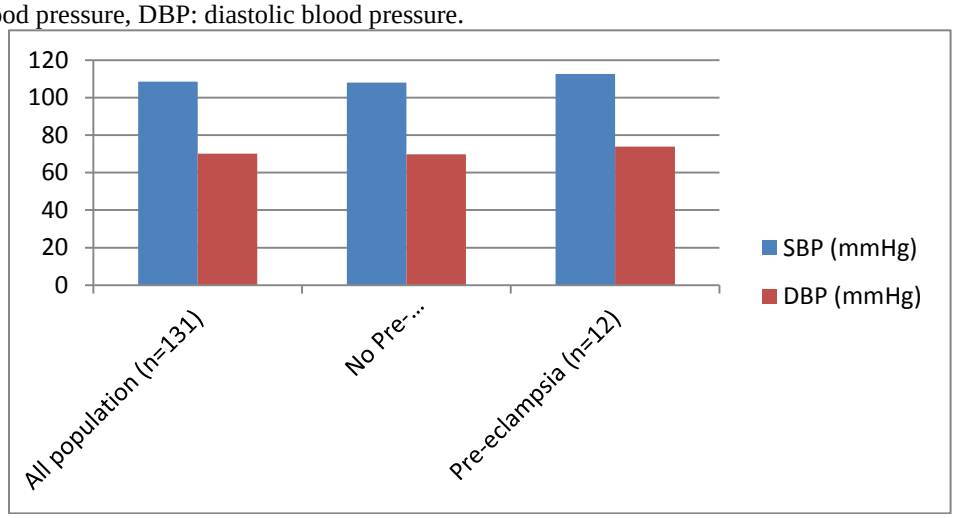


Figure (5): Blood pressure monitoring in patients with or without pre-eclampsia.

There was statistically highly significant difference between the two groups regarding serum C3 level as p value <0.05, with lower levels of C3 serum levels in preeclampsia group.

Table (4): Serum C3 level in the study population at first visit:

	All population		No Pre-eclampsia		Pre-eclampsia		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**p-value**
Serum C3 level (mg/dl)	146.5	39.6	155.4	33.8	58.3	19.1	**<0.001***

**highly significant.

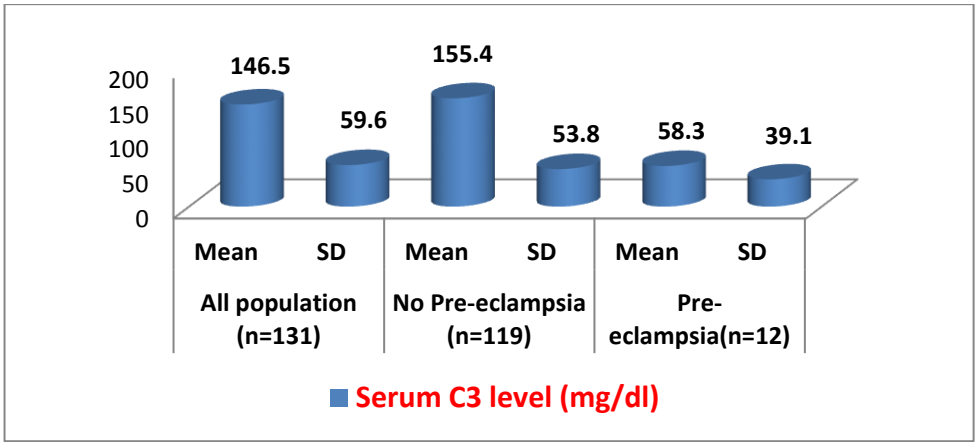


Figure (6): Mean serum C3 level in patients with or without pre-eclampsia. Error bars represent 95% CI.

There was highly significant statistical difference between the two groups as regard right uterine artery pulsatility index, resistance index at both sides as p value <0.001 and significant statistical difference as regard left uterine pulsatility index as p value <0.05 with higher PI and RI in the preeclampsia than the no preeclampsia group.

Table (5): Uterine artery Doppler indices in the study population at first visit.

	Total population (n=131)		No pre-eclampsia (n=119)		Pre-eclampsia (n=12)		
Variable	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**p-value**
Right uterine artery RI	0.60	0.10	0.58	0.07	0.81	0.07	**<0.001****
Left uterine artery RI	0.59	0.09	0.57	0.08	0.75	0.10	**<0.001****
Mean uterine artery RI	0.59	0.09	0.57	0.07	0.78	0.06	**<0.001****
Right uterine artery PI	1.11	0.11	1.01	0.21	2.10	0.58	**<0.001****
Left uterine artery PI	1.06	0.34	1.00	0.23	1.68	0.11	**0.003***
Mean uterine artery PI	1.09	0.34	1.01	0.19	1.89	0.14	**<0.001****

* significant
**highly significant.

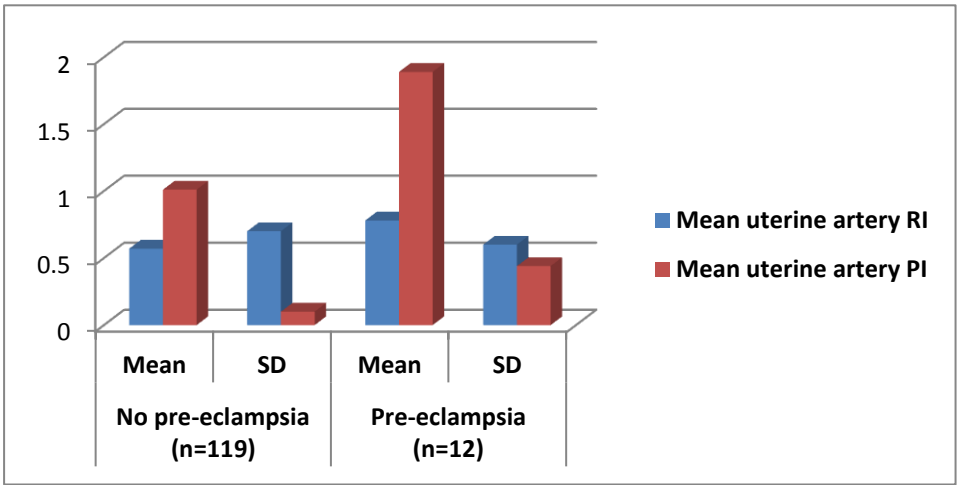


Figure (7): Mean uterine artery RI in patients with or without pre-eclampsia. Error bars represent 95% CI.

Table (6): Presence of diastolic notches on uterine artery Doppler in the study population.

Variable	Total populatio	No pre-eclampsia	Pre-eclampsia	p-value
Right uterine artery diastolic notch	6 (4.6%)	0 (0.0%)	6 (50.0%)	**<0.001****
Left uterine artery diastolic notch	5 (3.8%)	0 (0.0%)	5 (41.7%)	**<0.001****
Diastolic notch on any side	6 (4.6%)	0 (0.0%)	6 (50.0%)	**<0.001****

**highly significant

This table shows there was a significant association between the presence of diastolic notch and the development of pre-eclampsia.

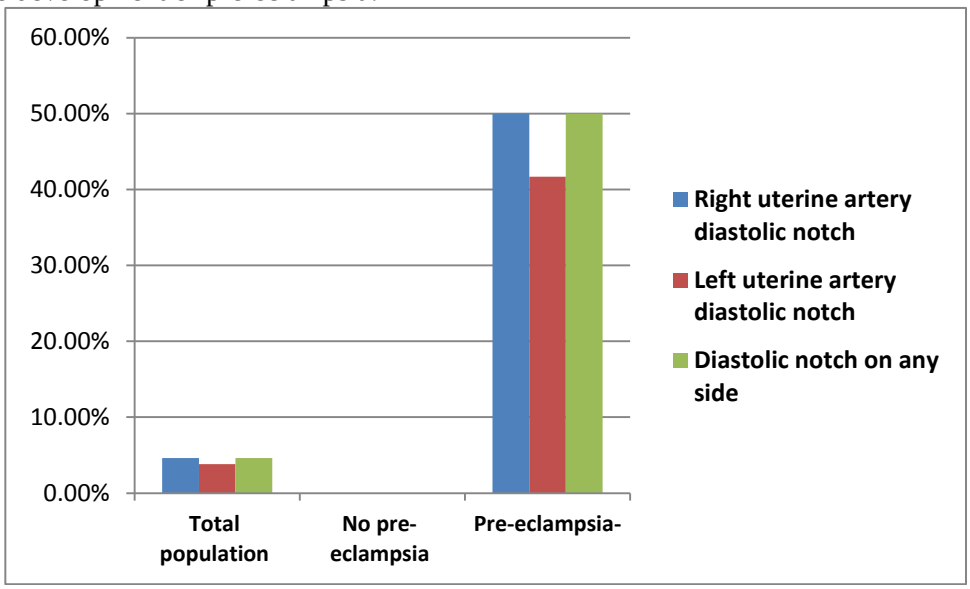


Figure (8): Prevalence of diastolic notches on uterine artery Doppler in the study population.

Receiver operator characteristics (ROC) curves were constructed for estimating the association between pre- eclampsia and mean uterine artery PI. A significant association was found with uterine artery PI being a significant predictor with higher values in preeclampsia cases than normal cases [area under the curve (AUC) = 0.99, 95% CI (0.954 to 0.999), best cut off(>1.35), sensitivity of100 %, specificity of 94.1%positive predictive value (PPV) of 63.2% and negative predictive value (NPV) of 100%.

Table (7): Receiver-operating characteristic curve analysis for classification of patients into those with or without pre-eclampsia using mean uterine artery PI.

	Estimate	95% CI	p-value
Area under the ROC curve (AUC)	0.990	0.954 to 0.999	**<0.0001****
Youden index J	0.9412		
Associated criterion	>1.35		
Sensitivity, %	100	73.5 - 100.0	
Specificity, %	94.12	88.3 - 97.6	
Positive predictive value (+PV), %	**63.2**	**38.4 - 83.7**	
Negative predictive value (-PV), %	**100**	**96.8 - 100.0**	

**highly significant

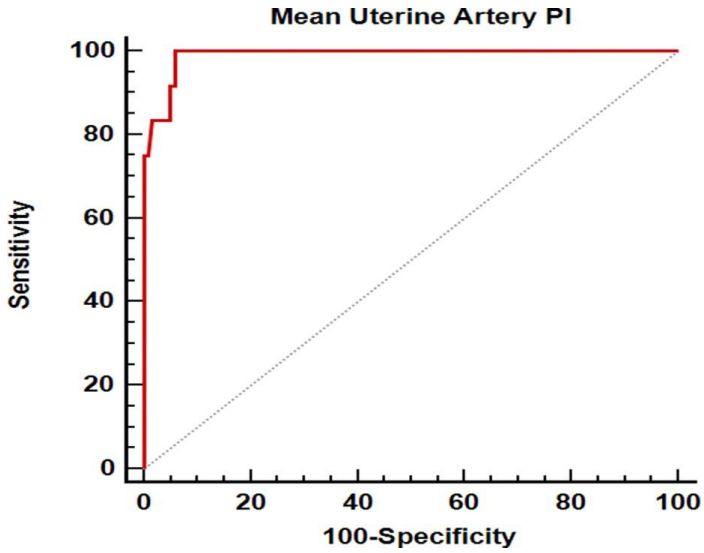


Figure (9): Receiver-operating characteristic curve for classification of patients into those with or without pre-eclampsia using mean uterine artery PI.

DISCUSSION

Pre-eclampsia is a complex multisystem disease that contributes significantly to maternal and neonatal mortality [(5).]

The classical clinical manifestations, *de novo* hypertension and proteinuria, occur late in pregnancy, in the setting of maternal endothelial cell activation [(6)] and excessive systemic inflammation [(7)].

The pathologic process originates in the placenta, with inadequate cytotrophoblast invasion in early pregnancy [(8)] leading to an oxidatively stressed placenta [(9)].

The immune system, a potent initiator of inflammatory pathways, is thought to play an important role in the etiology of pre-eclampsia [(10)].

In our study 9 cases are missed and 131 cases completed the study, 12 cases developed preeclampsia .While the rest of the cases are considered normal. There were no statistical significant difference between the normal and preeclampsia group regarding the mean maternal age. Also there were no statistical significant difference between the two groups in gestational age at enrollment, weight, height, SBP and DBP at first visit, While we found a statistical significant difference between normal and preeclampsia group regarding to BMI and regarding to gestational age at delivery. There were 9 cases developed proteinuria with incidence 75% of cases of preeclampsia.

Regarding C3 serum levels in the normal and preeclampsia groups, it was significantly lower in the preeclampsia group. So it was observed that the serum level of C3 was low in patients who develop preeclampsia in comparison with other patients who did not develop it.

In our study receiver operator characteristic (Roc) curves were created to demonstrate the

prognostic value of maternal serum C3 level at 14-20 week of gestation regarding to the development of pre-eclampsia with AUC (0.935 and p value<0.0001) and a cut-off value of mean serum C3 < 53.1mg/dl with sensitivity 83.3% and specificity 100%, PPV 100% and NPV 98.3%.In addition there is no significant correlation between serum C3 and the other variables.

Elevated C3a as early as the first trimester of pregnancy is an independent predictive factor for adverse pregnancy outcomes, suggesting that complement-related inflammatory events in pregnancy contribute to the subsequent development of poor outcomes at later stages of pregnancy **(11).**

This study coincides with study done by ***Aram and Shahbazi*** which was carried on 23 pre-eclamptic cases and 34 controls. In this study, the level of C3 & C4 in pre-eclamptic patients was lower than in those without pre- eclampsia. It is suggested that decreased level of these complements can be considered as a predictive factor of pre-eclampsia**(12)**.

Receiver operator characteristic (Roc) curves were created to demonstrate the prognostic value of (RI) and (PI) of left and right uterine arteries at 14 - 20 weeks of gestation regarding to the development of pre-eclampsia as for mean RI (AUC=1.0 and p value <0.0001), best cut off value=0.72 and at this value it has 100% sensitivity, 99.1% specificity,92.3% PPV and 100% NPV. As regards mean arterial PI (AUC=0.990 and p value <0.0001), best cut off value=1.35 and at this value it has 100% sensitivity, 94.1% specificity,63.2% PPV and 100% NPV. In addition there were statistically significant positive correlations between mean RI and mean PI and each other and highly statistical significant negative correlation with gestational age at delivery and no correlation with the other variables.

This goes with the work of ***Yu and colleagues*** **(13)** who detected similar results with statistically significant correlation (P value<0.001). As well ***Bodovaa and colleagus*** **(14)** stated the presence of statistically significant relation (P value <0.005) between the uterine artery changes on Doppler assessment at 24 weeks of pregnancy and the development of PE.

the sensitivity of RI to predict PE was 50% and specificity of 75%, and concluded that the uterine artery Doppler could be useful to detect pregnancy outcomes as PE and IUGR, bilateral evidence of notching and RI >0.72 is associated with adverse pregnancy outcome to improve the prediction of such changes uterine artery Doppler changes should be combined with biochemical testing as α- fetoprotein.

In the work of ***Pongrojpaw and colleagues,*** the sensitivity of PI to detect PE was 81.4% and the specificity 48.8% and so stressed that there should be a combination of uterine artery Doppler and biochemical testing as stated before **(15)** .

In the present study early diastolic notch was recorded in six patients (50%) which was bilateral in five cases and unilateral in one case in the preeclampsia group which was statistically significant as P value was <0.001, ***Yong Wan Park*** showed also that the presence of an early diastolic notch in uterine artery Doppler changes suggests high impedance vascular flow, moreover he categorized the notch according to its depth and observed adverse pregnancy outcomes with the group having deeper notch and hence concluded that it is not the presence of early diastolic notch merely, but its depth is the most important predictor for adverse pregnancy outcomes **(16)**.

CONCLUSION

This study demonstrates that lower level of maternal serum C3 in the early second trimester (14-20 weeks gestation) and abnormal increasing in uterine artery indices (PI and RI) are associated with developing pre-eclampsia several months later in pregnancy.

The study recommends that maternal combined maternal serum C3 and Doppler evaluation of the uterine arteries should be used in second trimester scan in patients at risk of preeclampsia.

Further studies on large number of cases are needed to confirm the current study results as we couldn't calculate the study odd's ratio.

RECOMMENDATIONS

The study recommends that maternal combined maternal serum C3 and Doppler evaluation of the uterine arteries should be used in second trimester scan in patients at risk of preeclampsia.

Further studies on large number of cases are needed to confirm the current study results as we couldnot calculate the study odd's ratio.

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