

## **Pregnancy-induced low serum Ficolin levels may underlie the development of Pre-eclampsia and predict it**

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Keywords: pre-eclampsia, Ficolins, Prediction of pregnancy & severity, Fasting blood glucose.

### **Abstract**

**Objectives:** Estimation of serum ficolin-2 and -3 for normotensive pregnant women to find a relation between ficolin levels and development of PE.

**Patients & Methods:** Primigravida attending the clinic prior to the 12<sup>th</sup> gestational week underwent blood pressure (BP) measurements and gave blood sample for estimation of fasting blood glucose (FBG) and ELISA estimation of serum ficolin. Enrolled women were asked to attend 4-weekly for BP measurement; 59 PE women (Group PE), 59 normotensive pregnant women (Group NT) and 59 non-pregnant women (Group NP) were enrolled.

**Results:** At time of PE diagnosis, all pregnant women had higher BP than their baseline measures, with significantly higher measures in PE women. Serum ficolin-2 levels were significantly lower in pregnant than NP women and in PE than NT women, while serum ficolin-3 levels were significantly lower in PE than NT women. Development of PE was positively associated with higher BMI, FBG and BP, while was negatively correlated with ficolin levels. Regression analysis defined low serum ficolin levels as negative predictors for PE development and serum ficolin-2 level <4.793 ng/ml can predict women liable to develop PE with 100% sensitivity and exclude PE with 100% negative predictive value.

**Conclusion:** Pregnancy has deleterious effect on complement pathway manifested by lower serum ficolin. Low serum ficolin-2 early in pregnancy is a sensitive screening test for pregnant women and can exclude PE development with 100% negative predictive value at level <4.793 ng/ml.

**Keywords:** Pre-eclampsia, Ficolins, Prediction of frequency & Severity, Fasting blood glucose

### **Introduction**

Preeclampsia (PE) is one of the most frequent and difficult illnesses in pregnancy, which jeopardizes both mother and fetus <sup>(1)</sup>. Clinically, PE is characterized by new onset maternal hypertension and proteinuria about the 20<sup>th</sup> gestational week (GW) in a pregnant woman who was normotensive prior to or at early pregnancy <sup>(2)</sup>.

The incidence of PE remains high and because its etiology and pathophysiology are still poorly understood, its management has not been established yet <sup>(3)</sup>. An intact complement system optimizes placental development and function and is essential to maintain host defense and fetal survival <sup>(4)</sup>. Also, significant and intricate immune adaptations are essential for the establishment and maintenance of normal pregnancy <sup>(5)</sup>.

Altered immune response may play part in PE pathogenesis <sup>(6)</sup>, dysregulation of complement Bb activation between 10<sup>th</sup> and 20<sup>th</sup> GW was reported in women who later on develop PE <sup>(7)</sup>, also genetic variations in complement genes C6 and Mannan-binding lectin-associated serine proteases 1 (MASP1) were found to be associated risk of PE and this risk varied by PE subtypes <sup>(6)</sup>.

Ficolins; L-, M-, and H-ficolin [Ficolin-1, Ficolin-2 and Ficolin-3, respectively) are soluble oligomeric defense proteins with lectin-like activity<sup>(8)</sup> and are structurally similar to the human collections, MBL and surfactant protein A and D<sup>(9)</sup>. Ficolins are present in human serum and differ in carbohydrate-binding specificity, but in common have the ability to recognize the acetyl group<sup>(10)</sup>. Ficolins can activate the lectin pathway of the complement system which provides innate immune protection against pathogens, marks host cellular debris for clearance, and promotes inflammation<sup>(11)</sup>. Ficolins are innate pattern recognition receptors and play integral roles within the innate immune response within organs and throughout the circulation to numerous pathogens<sup>(12)</sup>.

### **Hypothesis**

The current study hypothesized a relation between disturbed serum ficolin-2 and -3 levels and development of PE and so could be used as predictors for PE development and/or its severity.

### **Objectives**

Estimation of serum ficolin-2 and -3 early in pregnancy in women who are normotensives and follow-up for development of PE in trial to find a relation between early levels of ficolin and incidence and severity of PE

### **Design**

Prospective comparative multi-center clinical trial

### **Setting**

University Hospitals, Benha & Tanta, Egypt

### **Patients & Methods**

All primigravida attending the Antenatal Care Unit (ACU) of Benha University Hospital since May 2017 for assurance of being pregnant were evaluated for eligibility for inclusion in the study. All women underwent clinical evaluation and those with infectious diseases, inflammatory states, manifest diabetes, endocrinopathy, essential hypertension, renal, hepatic or cardiac diseases were excluded. Also, women with family history of essential hypertension, metabolic syndrome, or gestational hypertension were excluded from the study. During clinical examination body weight and height were determined and body mass index (BMI) was calculated as weight divided by squared height and only women with BMI < 35 kg/m<sup>2</sup> were included in the study. All enrolled women were asked to sign written fully informed consent to attend the ACU 4-weekly till delivery for follow-up. Women presenting after the 12<sup>th</sup> gestational week (GW), refused to sign the consent, or lost during follow-up were excluded.

### **The study protocols**

At time of presenting to ACU and after assurance of pregnancy, gestational age was determined and all women underwent full history taking and complete clinical examination including blood pressure (BP) measurements; systolic (SBP) and diastolic (DBP) while woman was in supine position. Enrolled women were asked to attend the ACU fasting for at least 8-hr on the start of the 12<sup>th</sup> GW to give fasting blood samples for routine and study investigations. Then, included women were asked to attend the clinic 4-weekly for measurement of SBP and DBP and evaluation of extent of proteinuria. This protocol was previously approved by the Local Ethical Committee.

## **Diagnosis and categorization of pre-eclampsia (PE)**

Preeclampsia (PE) was defined as development of gestational hypertension in a previously normotensive pregnant woman and is associated with proteinuria quantified as 1+ on dipstick<sup>(13)</sup>. PE was categorized as mild and severe according to BP measures obtained during follow-up visits, mild PE was diagnosed if SBP and DBP were <160 and <110 mmHg, respectively with proteinuria of <2+ and absence of systemic manifestations. Severe PE was diagnosed if elevated BP measures were associated with systemic manifestations or if SBP was  $\geq 160$  mmHg and DBP was  $\geq 110$  mmHg with proteinuria >2+ on a voided random urine<sup>(14)</sup>. Concerning timing of development of PE in relation to GW, PE was considered of early-onset if diagnosed prior to 34 GW and late if diagnosed after the 34<sup>th</sup> GW<sup>(15, 16)</sup>.

## **Groups**

1. Group PE included pregnant women who developed PE during pregnancy.
2. Group NT included number of pregnant women equal to that of PE women and were chosen from those who completed their pregnancy free of hypertensive manifestations.
3. Group NP included an equal number of non-pregnant women who were age-matched to women included in other groups and free of infectious or inflammatory disease as control group.

## **Laboratory investigations**

### **Blood sampling**

At the start of the 12<sup>th</sup> GW, all study participants gave 5 ml blood sample that was withdrawn under complete aseptic conditions, allowed to clot and then centrifuged at 3000 rpm for 10 minutes to separate serum that was collected in sterile Eppendorf tube and stores at -80°C till be assayed. Blood samples were collected and numbered by an assistant who was blinded about diagnosis.

### **Investigations**

Serum levels of ficolin-2 and ficolin-3 were measured using enzyme linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions and were read using a 96 well microplate ELISA reader (Dynatech. MR 7000).

1. Human ficolin-2 was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab213778, abcam, Cambridge, England) by quantitative sandwich enzyme immunoassay technique<sup>(17)</sup>.
2. Human ficolin-3 was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab213779, abcam, Cambridge, England) by quantitative sandwich enzyme immunoassay technique<sup>(18)</sup>.

### **Statistical analysis**

Obtained data were presented as mean $\pm$ SD, ranges, numbers and ratios. Results were analyzed using One-way Anova for analysis of variance between groups, paired t-test for analysis within each group and Chi-square test ( $X^2$  test) for analysis of non-numeric data. Sensitivity & specificity of studied parameters as predictors were evaluated using the receiver operating characteristic (ROC) curve analysis judged by the area under the curve (AUC) compared versus the null hypothesis that AUC=0.05. Regression analysis (Stepwise method) was used for stratification of studied parameters as specific predictors. Statistical analysis was conducted using the SPSS (Version 15, 2006) for Windows statistical package. P value <0.05 was considered statistically significant.

## Results

During the study period 491 primigravida attended the ACU for assurance of being pregnant; 73 women were excluded for not fulfilling the inclusion criteria and 418 women were enrolled in the study. During the pregnancy course 67 women developed PE and the remaining women completed their pregnancy free of PE manifestations. Unfortunately, 82 women were missed during follow-up; 8 PE and 73 normotensive women. Thus, 59 PE women had completed the observation period as Group PE and a similar number of normotensive women with cross-matched age and BMI were included as Group NT. A similar number of age and BMI cross-matched women were chosen from those who attended Family Planning Unit seeking for appropriate contraceptive method were chosen as Group NP (Fig. 1).

Revision of enrolment data of women of the three groups showed non-significant variance regarding age and height, while showed significant variance concerning patients' weight, BMI and fasting blood glucose. Interestingly, women who developed PE had significantly higher body weight, BMI and FBG in comparison to non-pregnant, while the differences were non-significant in comparison to normotensive pregnant women (Table 1).

Blood pressure measures estimated at time of enrolment showed non-significant variance between women of studied groups, while at time of diagnosis of PE showed significant variance between women of studied groups. At time of diagnosis of PE, all pregnant women showed elevated BP measures in comparison to their baseline measures, but the difference was non-significant in women of group NT, while was significant in PE women. Moreover, BP measures estimated at time of development of PE was significantly higher in PE women compared to corresponding measures of women of NT group and to women of NP group with non-significantly higher BP measures in women of group NT versus group NP (Table 2).

Eighteen women developed early PE; 6 had severe and 12 had mild PE, while 41 women developed late PE; 13 had severe and 28 had mild PE. Women developed early PE had SBP and DBP significantly higher compared to those had late PE and women developed severe PE had significantly higher BP measures than those had mild PE (Table 3).

Mean serum levels of studied parameters showed significant variance between women of studied groups. Mean serum levels of ficolin-2 were significantly lower in pregnant compared to non-pregnant women and were significantly lower PE than NT pregnant women. On contrary, serum ficolin-3 were significantly lower in PE women compared to NT and NP women of other groups, while were non-significantly higher in NT women than NP women (Table 4).

Presence of pregnancy was found to be positively associated with at enrolment increased body weight, BMI and higher serum FBG. Moreover, development of PE was found to be positively associated with higher at enrolment BMI, FBG and SBP. Estimated serum levels of ficolins were negatively correlated with presence of pregnancy and such correlation was significant with ficolin-2 ( $Rho=-0.392$ ,  $p=0.0007$ ), but was non-significant with ficolin-3 ( $Rho=-0.051$ ,  $p=0.498$ ). However, for development of PE, serum levels of both ficlon-2 ( $Rho=-0.353$ ,  $p=0.0003$ ) and ficlon-3 ( $Rho=-0.287$ ,  $p=0.002$ ) were negatively and significantly correlated with development of PE (Table 5).

Regression analysis of factors correlated with development of PE defined high at enrolment FBG ( $\beta=0.181$ ,  $p=0.020$ ) and SBP ( $\beta=0.267$ ,  $p=0.001$ ) as positive predictors, while low ficolin-2 ( $\beta=-0.440$ ,  $p=0.0005$ ) and ficolin-3 ( $\beta=-0.282$ ,

p=0.0009) as negative predictors for the possibility of PE development. ROC curve analysis defined high, at enrolment, FBG (AUC=0.617, p=0.028) and SBP (AUC=0.664, p=0.002) as specific predictors for oncoming PE, while low serum levels of ficolin-2 (AUC=0.297, p=0.0008) and ficolin-3 (AUC=0.334, p=0.002) as sensitive predictors for oncoming PE (Fig. 2).

Kaplan-Meier regression analysis defined a mean value for serum ficolin-2 at  $4.793 \pm 0.326$  ng/ml (95%CI: 4.153-5.432) as a cutoff point below which the hazard for PE development increases progressively, but was stationary at values above this cutoff point (Fig. 3). Evaluation of test validity character for this cutoff point, it showed 100% sensitivity for defining women liable to develop PE, 100% negative predictive value, 64.1% positive predictive value, 44.1% specificity rate and 72% accuracy rate.

Estimated serum level of ficolin-2 was negatively correlated with severity and timing of development of PE, but this correlation was non-significant (Rho: -0.195, p=0.138) with timing of PE development and significant (Rho: -0.573, p=0.0003) with severity of PE. ROC curve analysis showed that low serum ficolin-2 was specific predictor (AUC=0.752) for severe PE (Fig. 4).

## Discussion

The obtained results showed a positive association between pregnancy and high body weight and mass index (BMI) with high serum fasting blood glucose (FBG) early in pregnancy. These findings indicated that pregnancy itself is obesity inducing and glucogenic state and this could be attributed to the fact that pregnancy induced vicious circle of getting obese with subsequent induction of insulin resistance (IR) that mostly induce a picture of pre-diabetes even if the woman was not diabetic prior to pregnancy nor developed gestational diabetes.

Multiple recent experimental studies assured this assumption and tried to explain these changes, wherein **Chen et al.** <sup>(19)</sup> found that in normally pregnant animals apo-retinol-binding protein 4 activates the stimulated by retinoic acid 6 signaling cascade, inducing IR through decreased phosphorylation of insulin receptor and insulin receptor substrate 1, and attenuated GLUT4 translocation and glucose uptake. Also, **Petry et al.** <sup>(20)</sup> detected a link between pregnancy-associated plasma protein A concentrations in early pregnancy and subsequent glucose concentrations and blood pressures and attributed this to changes in insulin sensitivity and secretion. Thereafter, **Cardenas-Perez et al.** <sup>(21)</sup> found maternal programming by high-fat diet causes failure in glucose, leptin and insulin sensitivity and fat accumulation and **Olaniyi & Olatunji** <sup>(22)</sup> reported that obesity and hepatic lipid accumulation during pregnancy is accompanied by increased pyruvate dehydrogenase kinase-4

This study detected a positive significant correlation between high BMI, SBP and FBG at the 12<sup>th</sup> GW with the development of PE later during the pregnancy course. These findings spot light on the impact of pre-conception obesity and increased blood glucose level on blood pressure measures and subsequent PE development. In line of these data regression and ROC curve analyses defined elevated BMI and SBP early during pregnancy as significant predictors for later on development of PE.

These findings go in hand with **Falcone et al.** <sup>(23)</sup> who reported that bariatric surgery for obesity could ameliorate pregnancy-induced hypertension (PIH) and with **Kalafat et al.** <sup>(24)</sup> who found metformin therapy for gestational diabetes significantly reduced the risk of PIH. Moreover, **Davenport et al.** <sup>(25)</sup>, (2018) documented that exercise interventions during pregnancy effectively lowered the possibilities for

gestational diabetes, gestational hypertension and PE development. Recently, **Zhuang et al.**<sup>(26)</sup> reported that BMI was positively correlated with the occurrence of PIH and reduction of BMI may reduce the prevalence of this complication. Also, **Siddiqui et al.**<sup>(27)</sup> found severe antepartum hypertensive disorders were most strongly associated with obesity.

There was a negative significant correlation between development of PE and serum levels of ficolins. Moreover, statistical analyses reported that low serum level of ficolin-2 is a significant predictor of PE with 100% sensitivity and could exclude its future development with negative predictive value of 100%, especially if its serum level was >4.793 ng/ml which is a diagnostic cutoff point with high specificity as judged by area under ROC.

These findings supported earlier studies reported that mannose-binding lectin pathway activity is involved in pathogenesis of PE<sup>(28, 29, 30)</sup>. Also, **Halmos et al.**<sup>(31)</sup> detected significantly lower serum ficolin-2 and ficolin-3 levels in PE women than healthy pregnant women and significantly correlated with PIH-inducing angiogenic factors, plasma VWF: antigen, fibronectin and cell-free fetal DNA concentrations.

Recently, **Larsen et al.**<sup>(32)</sup> found H-ficolin, M-ficolin and MASP-3 serum levels of PE women were lower than in normotensive pregnant women, in decreasing order of significance and MASP-3 levels were increased after delivery in PE and normotensive women, while serum H-ficolin levels were significantly increased after delivery in PE women

Interestingly, the current study reported significantly lower serum ficolin-2 and non-significantly lower serum ficolin-3 in pregnant women than in non-pregnant women, a finding indicated an impact of pregnancy itself on complement system. This finding assured that previously reported by **Halmos et al.**<sup>(31)</sup> who detected significantly lower plasma levels of ficolin-2 in healthy pregnant than in healthy non-pregnant women, while ficolin-3 levels did not differ significantly between the two groups.

## Conclusion

Pregnancy has deleterious effect on complement pathway manifested by lower serum ficolins. Disturbed complement pathway has a role in pathogenesis of PE. Lower serum ficolin-2 early in pregnancy is a sensitive screening test for pregnant women and can exclude the development of PE with 100% negative predictive value at cutoff point of 4.793 ng/ml. However, wide scale multicenter study is mandatory to establish the value of this cutoff point

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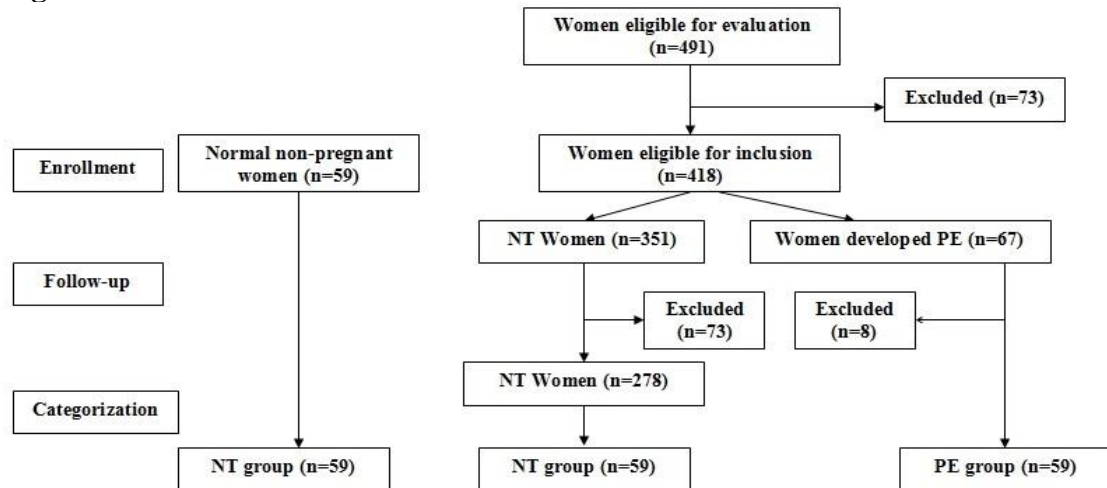
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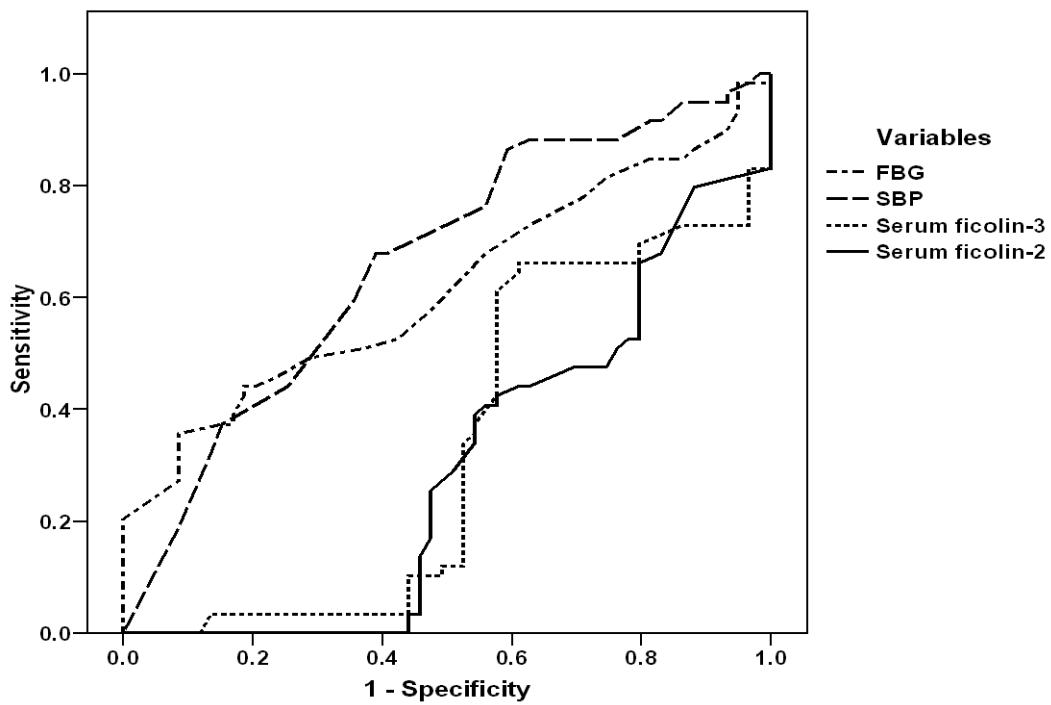
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**Figures:**



**Fig. (1):** Flow chart of the study



**Fig. (2):** showing ROC curve for studied variables as predictors for development of PE

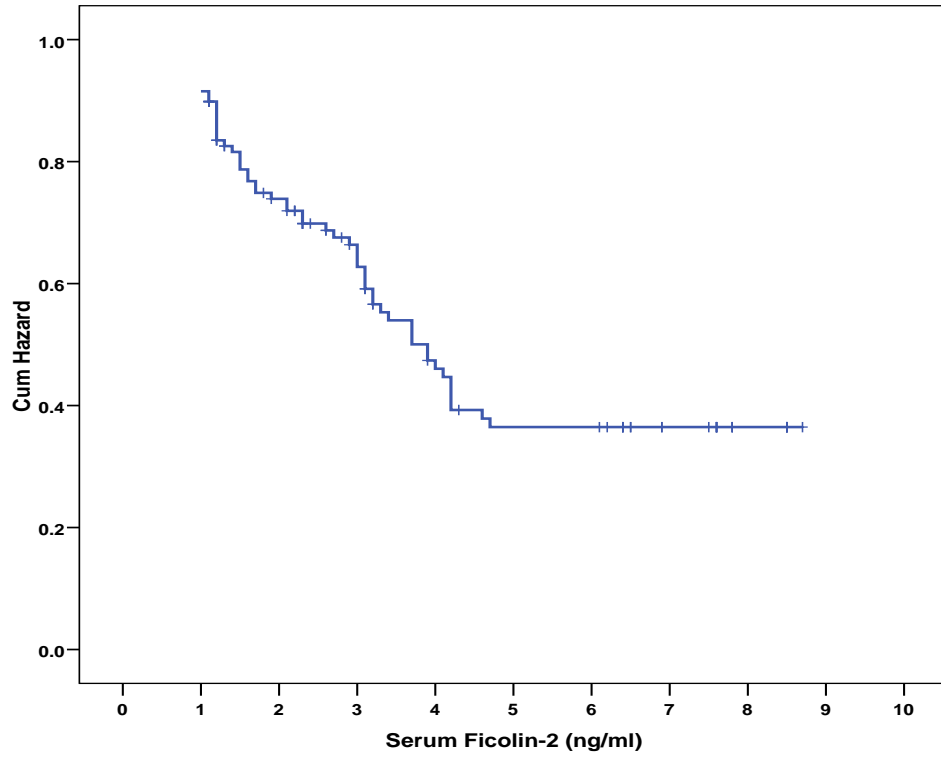


Fig. (3): showing cumulative hazard for developing PE at cutoff point for serum ficolin-2 of 4.793 ng/ml

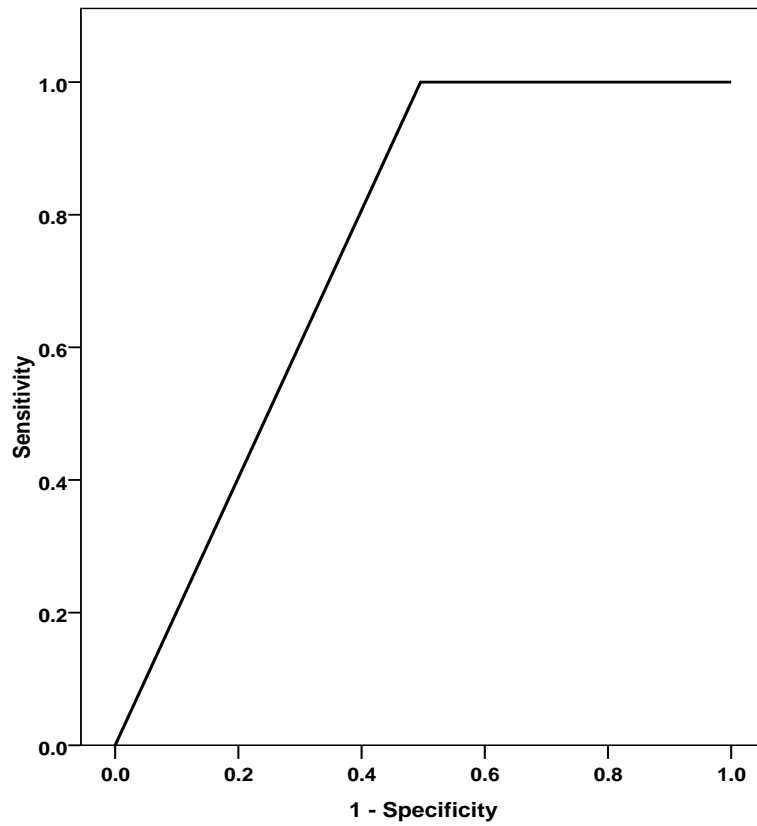


Fig. (4): showing AUC for serum ficolin-2 level as predictor for severe PE

**Tables:**

**Table (1): Enrolment data of studied patients**

Data		Group NP (n=59)	Group NT (n=59)	Group PE (n=59)	P
Age (years)		28±6.1	26.8±5.6	26.8±7	0.481
BMI	Weight (kg)	74.6±7.5	76±9	79.5±10.2*	0.012
	Height (cm)	169.8±2.1	170±2.4	169.6±2.6	0.653
	BMI (kg/m <sup>2</sup> )	25.9±2.9	26.3±3	27.6±3.4*	0.008
Fasting blood glucose (mg/dl)		87.2±6	90±9.2	92.3±11.8*	0.013

Data are presented as mean±SD; P value indicates significance of variance between groups; p>0.05 indicates non-significant difference; p<0.05 indicates significant difference; \* indicates significant difference versus NP women.

**Table (2): Blood pressure measures estimated at time of enrolment and development of PE manifestations compared corresponding levels in women of other groups**

Time of estimation		Group NP (n=59)	Group NT (n=59)	Group PE (n=59)	P
At enrolment	SBP (mmHg)	117.9±4.5	118±4.4	118.3±5	0.237
	DBP (mmHg)	82.2±4.9	82.9±3.6	83.4±3.5	0.298
At development of PE	SBP (mmHg)	118.2±4.5	119.5±8.8	154±11.4	<0.0001
	P1=		0.092	<0.0001	
	P2=		0.303	<0.0001	
	P3=			<0.0001	
	DBP (mmHg)	82.2±4.9	84.7±3.2	100.1±8.8	<0.0001
	P1=		0.361	<0.0001	
	P2=		0.077	<0.0001	
	P3=			<0.0001	

Data are presented as mean±SD; NP: Non-pregnant; NT: Normotensive; PE: Pre-eclampsia; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; P value indicates significance of variance of measures of the three study groups; P1 indicates significance of difference in comparison to respective at enrolment measures; P2 indicates significance of difference versus respective measures of Group NP; P3 indicates significance of difference of versus measures of Group NT; p<0.05 indicates significant difference; p>0.05 indicates non-significant difference.

**Table (3): Mean blood pressure measures of women developed PE, categorized according to timing of development of PE and its severity**

Variable	Category	Early PE (n=18)	Late PE (n=41)	Total (n=59)
SBP (mmHg)	Mild PE (n=40)	149±3.9	145.6±3.1*	146.6±3.7
	Severe PE (n=19)	172±3.9	168.4±2.8*	169.5±3.5**
	Total (n=59)	156.7±11.8	152.8±11.2*	154±11.4
DBP (mmHg)	Mild PE (n=40)	95.3±1.7	93.9±1.7*	94.3±1.8
	Severe PE (n=19)	115±2.6	111±3.7*	112.3±3.8**
	Total (n=59)	101.9±9.7	99.3±8.4*	100±8.8

Data are presented as mean±SD; PE: Pre-eclampsia; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; \* indicates significance of difference versus Early PE; \*\*: indicates significance of difference of versus Mild PE.

**Table (4): Mean levels of estimated parameters in blood samples obtained at time of enrolment**

Parameter		Group NP (n=59)	Group NT (n=59)	Group PE (n=59)	P
Ficolin-2	Level	5.66±2.6	4.46±2.8	2.34±1	<0.0001
	P1=		0.016	<0.0001	
	P2=			0.0006	
Ficolin-3	Level	24.69±8.5	26.8±14.6	20.1±10.7	0.0059
	P1=		0.339	0.0102	
	P2=			0.005	

Data are presented as mean±SD; NP: Non-pregnant; NT: Normotensive; PE: Pre-eclampsia; PIGF: Placental growth factor; sFlt-1; P value indicates significance of variance of levels estimated in women of the three study groups; P1 indicates significance of difference versus respective measures of Group NP; P2 indicates significance of difference of versus measures of Group NT; p<0.05 indicates significant difference; p>0.05 indicates non-significant difference.

**Table (5): Spearman's correlation between clinical and laboratory data determined at time of enrolment and presence of pregnancy and development of PE**

	Pregnancy		PE	
	Rho	p	Rho	P
Age	0.152	0.053	0.066	0.481
Body weight	0.261	<0.001	0.176	0.057
BMI	0.281	<0.001	0.206	0.025
FBG	0.195	0.009	0.204	0.027
SBP	0.045	0.554	0.286	0.002
DBP	0.015	0.839	0.131	0.159
Ficolin-2	-0.392	0.0007	-0.353	0.0003
Ficolin-3	-0.051	0.498	-0.287	0.002

BMI: Body mass index; FBG: Fasting blood glucose; SBP: Systolic blood pressure; DBP: Diastolic blood pressure