Deregulated Immune Milieu Early during Pregnancy could discriminate women vulnerable to develop Gestational Hypertensive Disorders

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Short title

Cytokines as early predictors for PE.

Abstract

Objective: To evaluate the potential of serum tumor necrosis factor-α (TNF-α), interleukin (IL)-6 and 10, and placental growth factor (PLGF) as early biomarkers for gestational hypertension (GHTN) or preeclampsia (PE) by measuring their serum levels at 12 gestational weeks (GW) and correlating them with blood pressure (BP).

Patients & Methods: BP was measured 4-weekly during pregnancy and women who developed significantly higher BP measures than the at-12th GW measures were considered as GHTN and were followed up, women who developed proteinuria were collected as the PE group, while the remaining were collected as the GHTN group and women who sustained normal BP measures till the 38th GW were collected as the normotensive (NT) group.

Results: Serum TNF-α and IL-6 levels were significantly elevated in PE (n=24) and GHT women (n=41) compared to NT women (n=65). PE women had significantly lower serum IL-10 and PLGF levels than both NT and GHT women. Elevated IL-6 and TNF-α demonstrated a high positive predictive value for the development of GHT. Conversely, low serum IL-10, PLGF, and a low IL-10/IL-6 ratio were identified as sensitive early predictors of PE. High serum TNF-α levels emerged as the most crucial early predictor of hypertension development before the 34th GW.

Conclusion: elevated serum levels of IL-6 and TNF-α at the 12th GW especially in obese pregnant women might identify women vulnerable to developing GHTN, while low serum levels of PLGF and IL-10 or IL-10/IL-6 ratio might function as early predictors for the development of PE.

Keywords: Gestational hypertension; Preeclampsia; Placental growth factor; Interleukins; Tumor necrosis factor-α.

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Introduction

Preeclampsia (PE) is a multifactorial pregnancy hypertensive disorder (PHD) that variably impacts both maternal and fetal health (1). Numerous studies have sought to identify new therapeutic agents for the prevention or treatment of PE (2); however, no treatment or marker is universally applicable to all case [(1)].

In a healthy pregnancy, the decidualization of uterine stromal cells is crucial for fetal implantation, local adaptation, and the growth and maintenance of the placenta [(3)]. Deficiencies in maternal decidualization can lead to adverse pregnancy outcomes, including PE [(4)]. Placentae from PE-affected pregnancies exhibit shallow implantation, inadequate spiral artery remodeling, and elevated expression of anti-angiogenic factors [(5)].

Placental growth factor (PLGF) is primarily expressed in the placenta, though it is also present at lower levels in various other tissues [(6)]. PlGF-1 and PlGF-2 are secreted during pregnancy in a closely correlated manner [(7)] and are key proteins for placental angiogenesis and vasculogenesis, particularly during embryogenesis [(8)].

The placenta in early pregnancy directly influences fetal development and the maternal immune system, leading to the release of inflammatory and immunomodulatory factors. However, it may also cause abnormal release of cytokines and chemokines in syncytiotrophoblasts [(9)].

During pregnancy, tumor necrosis factor-alpha (TNF-α) is a multifunctional Th1 cytokine that influences hormone synthesis, placental structure, and embryonic development [(9)]. TNF-α stimulates the production of IL-6, which subsequently promotes Th2 phenotypic responses. The actions of TNF-α can be both pro- and anti-inflammatory (10). The local balance between IL-6 and IL-10 is a critical determinant of subsequent immune responses [(11)].

Objective

Estimation of serum levels of inflammatory cytokines and human placental growth factor at the 12th GW and correlating these levels with blood pressure (BP) measures to define an early predictor for the development of gestational hypertension (GHT) and its progress to PE.

Design

Prospective comparative trial.

Setting

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Ethical consideration

The study protocol was approved by the Local Ethical Committee at Tanta Faculty of Medicine. Enrolled women provided informed consent to participate in antenatal care and blood sample collection. To maintain study integrity, participants were blinded to the specific investigations, and the obstetrician and biochemist were blinded to the study's purpose, participant demographics, and cytokine levels.

Patients & Methods

All women who attended the Antenatal Care unit, Tanta University Hospital from July 2019 to June 2022, for assurance of pregnancy were eligible for evaluation. Pregnancy was diagnosed chemically and assured clinically by US examination. At the time of 1st attendance to the clinic, full history taking was carried out to collect data including present and family histories of diabetes mellitus, essential hypertension, liver or kidney dysfunction, endocrinopathy. Obstetric history included several gravidity and parity, development of pregnancy-associated diseases as GHT, PE, gestational diabetes mel-

litus, kidney or liver diseases. Clinical evaluation included a collection of demographic data including age, body weight, and height, and calculation of body mass index (BMI) as weight divided by the square value of height in meters to give BMI in kg/m2 (Bray, 1992). BMI (kg/m^2) was graded according to WHO guidelines (WHO, 1994) as average weight (BMI<24.9 kg/m^2), overweight (BMI=25-<30 kg/m^2), and obese (BMI=30-<35 kg/m^2) and morbid obese women (BMI>35 kg/m^2). Then, all women had a general examination with estimation of systolic and diastolic blood pressure (SBP & DBP) to ensure that the enrolled women were normotensive at the time of enrolment. Then, blood samples were obtained from all women for routine lab investigations.

Exclusion criteria

Women were excluded from the study if they had multiple pregnancies, fetal abnormalities, pre-existing diabetes, hypertension, renal, hepatic, or cardiac disease, or endocrine disorders, particularly those associated with obesity. Women who were lost to follow-up or missed scheduled BP measurements were also excluded.

Inclusion criteria

Pregnant women with singleton pregnancies, diagnosed at Tanta University Hospital's ANC unit, and meeting all inclusion criteria were eligible for the study. Participants provided written informed consent to attend scheduled clinic visits and donate blood samples for required investigations.

Diagnosis and categorization of pre-eclampsia (PE)

- SBP and DBP that were measured at the enrolment of the study were considered as the comparative baseline measures. Blood pressure measurements were taken every 4 weeks throughout the pregnancy.
- According to the American Society of Hypertension
- Gestational hypertension (GHTN) is defined as the development of HTN after the 20th GW in a previously normotensive (NT) woman at the time of pregnancy diagnosis [12].
- Preeclampsia was diagnosed as the development of GHT accompanied by quantifiable proteinuria (1+ on dipstick) in a previously normotensive pregnant woman[12].
- Preeclampsia severity was classified according to the American College of Obstetricians and Gynecologists guidelines (13). Mild preeclampsia (MPE) was defined by systolic blood pressure (SBP) < 160 mmHg, diastolic blood pressure (DBP) < 110 mmHg, proteinuria ≤ 2+, and the absence of systemic symptoms. Severe preeclampsia (SPE) was characterized by elevated blood pressure with systemic manifestations or SBP ≥ 160 mmHg, DBP ≥ 110 mmHg, and proteinuria > 2+ on a random urine sample.
- Timing of Onset: Preeclampsia was categorized as early-onset (EPE) if diagnosed before 34 weeks of gestation (GW) and late-onset (LPE) if diagnosed after 34 GW [14, 15].

Grouping

- Women who had BP that was non-significantly higher in comparison to measures obtained at the 12th gestational week (GW) with or without proteinuria of 1+ on urinary strips were considered normotensive women. For comparative purposes, several NT women of BMI cross-matched with those of the HT group were randomly collected as NT group.
- Women who developed hypertensive manifestations defined as significantly higher BP follow-up measures in comparison to measures obtained at the 12th GW with or without proteinuria of 1+ on urinary strips were grouped as hyperten-

sive (GHT) group

- Women of the GHT group were followed up 4-weekly for estimation of BP and evaluation of proteinuria, those who progressed to PE were collected as PE group and were differentiated as early or late and according to severity into mild or severe.

Laboratory investigations

Blood sampling

Blood samples (5 ml) were collected at the onset of the 12th gestational week under strict aseptic conditions. The samples were left to clot and subsequently centrifuged at 3000 rpm for 10 minutes to separate the serum. The isolated serum was then transferred to a sterile Eppendorf tube and stored at -80°C until further analysis. An assistant, unaware of the group assignments, performed the collection and numbering of the blood samples.

Investigations

Serum levels of human tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-10 (IL-10), and placental growth factor (PLGF) were measured using enzyme-linked immunosorbent assay (ELISA) kits. A 96-well microplate ELISA reader (Dynatech MR 7000) was used to quantify the results.

1. TNF-α: A Pelikine™ Inc. ELISA kit (Concord, USA) was used to measure human serum TNF-α levels [16].
2. PLGF: Human placental growth factor (PLGF) was measured using a quantitative sandwich enzyme immunoassay technique with an R&D Systems Inc. ELISA kit (Minneapolis, USA, catalog no. DPG00 SPG00 PDPG00) [17].
3. IL-10: Human serum IL-10 levels were determined using a Milenia® ELISA kit from DPC Biermann (Bad Nauheim, Germany) [18].
4. IL-6: A Pelikine™ Inc. ELISA kit (Concord, USA) was used to measure human serum IL-6 levels [19].

Study outcomes

1. Primary outcome: the ability of the estimated serum cytokines at the 12th GW to discriminate pregnant women vulnerable for the development of GHT and progress to PE.
2. The importance of the variables obtained at the 12th GW as predictors for high BP at the 34th GW as a cutoff time for differentiation between early and late PE [14, 15].

Statistical analysis

Data were presented as mean ± SD, numbers, percentages, median, IQR, or Chi-square test, as appropriate. Paired t-tests, one-way ANOVA with Tukey's HSD post-hoc test, or Spearman's rank correlation coefficient were used for comparisons. ROC curve analysis evaluated biomarker predictive ability. An automated linear model identified key biomarkers for early PE risk. IBM SPSS Statistics (Version 23, 2015) was used for statistical analyses. A p-value < 0.05 indicated statistical significance.

Results

Of the 391 eligible pregnant women, 319 were enrolled after excluding 26 who did not meet the inclusion criteria and 46 who were lost to follow-up. All participants experienced BP fluctuations during pregnancy. A total of 65 women developed HT, with 24 progressing to PE and 41 remaining as GHT (Fig. 1). Baseline characteristics of the enrolled women were not significantly different ($p > 0.05$). Patients' distribution according to the BMI grade showed insignificantly higher frequency of obese women among HTN and PE women than among NT women (Table 1).

At the 12th and 16th GW, both SBP and DBP measures showed non-significant ($p>0.05$) differences between the three groups. Blood pressure measures of women of the NT group showed non-significant ($p>0.05$) differences

in comparison to their corresponding 12th GW measures throughout the pregnancy duration. Women of the GHT group showed a progressive increase in their BP measures from the 20th till the 38th GW. Systolic and diastolic BP measures of women of the GHT group were significantly higher than the corresponding measures of women of NT at the 34th and 38th GW and the differences were significant in comparison to their 12th GW measures. On contrary, BP measures of women of the PE group were significantly higher in comparison to the corresponding 12th GW measures throughout the pregnancy course and measures of women of NT and GHT groups during the duration of 20-38 GW (Table 2, Fig. 2).

All enrolled women had no proteinuria till the 12th GW; at the 16th and 20th GW 31 and 40 women, respectively had proteinuria of +1 on dipstick with significantly lower frequency of developing proteinuria among women of NT group in comparison to women of GHT ($p=0.027$ and 0.022, respectively) and PE ($p=0.014$ and 0.0001, respectively) groups and non-significantly ($p=0.634$ and 0.089, respectively) higher frequency among women who developed PE. All patients of PE women developed proteinuria since the 28th GW till the end of pregnancy, while patients of NT and GHT groups showed a stepwise increase in the frequency of getting proteinuria with non-significantly higher frequency among women of GHT in comparison to those of NT group till the 34th GW, while the difference was significant ($p=0.019$) at the 38th GW (Table 3).

Serum TNF-α and IL-6 levels were significantly higher at the 12th gestational week in women who later developed GHT ($p = 0.00009$ and <0.0001, respectively) or PE ($p < 0.0001$ for both) compared to normotensive women. In contrast, serum IL-10 and PLGF levels were significantly lower in women who developed GHT ($p < 0.0001$) and PE ($p < 0.0001$) compared to normotensive women.

Furthermore, women with PE had significantly higher serum TNF-α and IL-6 ($p = 0.0022$ and 0.0317, respectively) and significantly lower serum IL-10 ($p = 0.0001$) and PLGF ($p = 0.0196$) levels compared to those with GHT. Consequently, the calculated IL-10/IL-6 ratio was significantly lower in women with PE compared to both NT and GHT women, with a significantly lower ratio also observed in GHT women compared to NT women (Table 4, Fig. 4).

The development of GHT and PE was positively correlated with higher BMI, serum TNF-α, and IL-6 levels. Conversely, it was negatively correlated with serum IL-10 and PLGF levels. Furthermore, SBP measurements between the 20th and 38th gestational weeks showed a positive correlation with serum TNF-α and IL-6 levels, while a negative correlation was observed with serum IL-10 and PLGF levels (Table 4).

Analysis of variables at the 12th GW identified high serum levels of IL-6 and TNF-α, as well as high BMI, as strong early predictors for the development of GHT during pregnancy (Fig. 3). Conversely, low serum levels of IL-10 and PLGF, and a low IL-10/IL-6 ratio, emerged as sensitive early predictors for the development of PE (Table 5, Fig. 4).

Stratification of the variables defined by ROC curve analysis using the automatic linear analysis as early predictors of HT development before 34th GW defined high serum levels of TNF-α by 54%, followed by low serum IL-10 levels by 33% and high BMI as the least important predictor by 13% (Fig. 5).

Discussion

All pregnant women even those who continued their pregnancy with BP measures within normal ranges showed increased BP measures compared to their baseline measures. These findings indicated that pregnancy per se is a hypertensive process even if associated with minimal elevation of BP measures. Moreover, the obtained results showed vari-

able body response to the hypertensive effect of pregnancy, wherein some women had progressed to PE with shooting up BP measures to >170/110 mmHg, while others had BP measures had elevated up to 140/93 mmHg. These findings indicated the presence of certain modulators of BP in direction of hypertension with a varied individual effect.

In support of these data and assumptions, the current study detected significantly lower serum PLGF levels in studied women that were negatively correlated with the development of GHT and PE. Such relation could be attributed to previously documented that placental growth factor which is an angiogenic factor that was required for proper placentation and its decreased levels are related to pregnancy-associated cardiovascular complications that were proved to be related to cardiovascular events later in maternal life [8, 20]. In line with the reported lower serum PLGF and its relation to the development of pregnancy-related hypertensive complications, Infante et al. [21] detected significantly lower PLGF levels in GHT than in normal pregnancies, and Andersen et al. [22] reported an inverse relationship between serum levels of PLGF and hypertension and supposed 66 as the optimal single ratio between anti-angiogenic/angiogenic factors (sFlt-1/PLGF) for prediction of PE especially late-onset type. Also, it was found that in women with chronic hypertension and superimposed PE, lower serum PLGF and high sFlt-1/PlGF ratio are predictive of delivery because of maternal or fetal indications [23], and maternal serum angiogenic markers were found to be superior to clinical assessment in predicting adverse maternal and perinatal outcomes in pregnant women with chronic hypertension [24].

Serum cytokines' levels estimated at the 12th GW indicated immune milieu deregulation in direction of inflammation as evidenced by increased serum levels of inflammatory cytokines (TNF-α and IL-6) and decreased serum level of the anti-inflammatory cytokine, IL-10 with subsequent inversion of anti/pro-inflammatory ratio; thus, indicating increased inflammation. Similarly, Mtali et al. [25] found neutrophil counts and levels of IL-6 were significantly higher in women with hypertensive pregnancy disorders than in those with normal pregnancy and IL-6 levels positively correlate with the severity of hypertension. Also, Žák & Souček [26] reported a significant positive correlation between serum levels of TNF-α and IL-6 and blood pressure; systolic in the 2nd and diastolic in the 2nd and 3rd trimesters with a significant positive association between high TNF-α and risk of PE in the 3rd trimester. Recently, Aldika Akbar et al. [27] detected significantly higher serum levels of IL-6 and endothelin-1 with lower NO levels in women with PE.

Correlation analyses revealed a positive association between serum levels of TNF-α and IL-6, while a negative association was observed between serum levels of IL-10 and the development of GHT or PE. ROC curve analysis further confirmed these findings, identifying high TNF-α, IL-6, and BMI as significant predictors of GHT and PE, and low serum IL-10 as a sensitive indicator for these complications.

These results align with previous research by Zhou et al. [28], who reported significantly increased relative expression of TNF-α mRNA and TNF-α protein levels in women with severe PE. Additionally, they found a positive correlation between placental TNF-α expression and systolic and diastolic blood pressure.

Moreover, there was a negative significant correlation between serum levels of TNF-α and IL-6 and that of PLGF; thus, indicating the presence of a vicious circle consisting of the deregulated immune system, antiangiogenic/angiogenic system, and obesity leading to gestational hypertensive complications. Multiple trials tried to explore the pathogenic basis for the relation between these deregulated systems and BP during pregnancy. Akasaka et al. [29] on exposure of human primary cultured adipocytes with serum from women with gestation hypertensive diseases for

24 h, detected significantly higher levels of mRNA of IL-6, C-C motif chemokine ligand 2 (CCL2), and RAGE system (advanced glycation endproducts and its receptors)and suggested that the elevated advanced glycation endproducts and high mobility group box up-regulated the expression of IL-6 and CCL2 leading to systemic inflammation causing hypertensive disorders in pregnant women. Using the PE rat model, Wang et al. [30] detected an increased expression level of serum IL-6 and upregulation of the protein expressions of phosphorylated STAT1 with increased oxidative stress and apoptosis of placental trophoblastic cells. Askin & Andan [31] documented that obesity decreases serum levels of protein 9 of the complement C1q TNF-associated protein, which has a strong vasorelaxation effect with the adiponectin receptor-1/NO signaling pathway, thus contributing to the pathogenesis of PE with adverse effects on the vascular and placental system.

Experimental animal models, such as the reduced uterine perfusion pressure rat model of PE, have provided valuable insights into the role of inflammation in the pathogenesis of PE. Travis et al. [32] demonstrated that TNF-α blockade reduced mean arterial pressure (MAP) and uterine artery resistance index while increasing NO bioavailability, suggesting a potential role for TNF-α in regulating NO bioavailability and contributing to PE pathophysiology.

Similarly, Huang et al. [33] observed increased MAP, elevated serum levels of TNF-α, IL-6, and sFlt-1, and decreased levels of vascular endothelial growth factor, nitrite, and nitrate in a reduced uterine perfusion pressure rat model. These changes were attributed to the down-regulation of silent information regulator-1, leading to inhibition of the Sonic Hedgehog pathway.

Zhang et al. [34] further investigated the role of sphingosine-1 phosphate receptor-2 (S1PR2) in PE pathogenesis. They found significantly increased placental expression of S1PR2, along with elevated serum and placental levels of inflammatory cytokines (TNF-α, IL-1β, and IL-6) and decreased expression of VEGF and increased expression of FMS-like tyrosine kinase 1 in placental tissue, suggesting a potential link between S1PR2 and the development of PE.

Conclusion

Pregnancy is a hypertension-inducing condition but to a variable extent. Such an effect could be attributed to the deregulation of the immune milieu with increased serum levels of inflammatory cytokines and decreased levels of anti-inflammatory cytokines. High serum levels of IL-6 and TNF-α, in association with high BMI, might identify women vulnerable to developing GHTN, while low serum levels of IL-10 and PLGF, and a low IL-10/IL-6 ratio might function as early predictors for the development of PE.

Limitation

Estimation of serum levels of anti-angiogenic factors for evaluation of the impact of deregulation of angiogenic/anti-angiogenic system on the pathogenesis of GHT disorders.

Recommendation

Wider scale study to evaluate the effect of preconception weight reduction on the estimated cytokines and the incidence of GHT disorders.

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Table (1): Baseline data determined at the 12th week GA

Data	Group		Normotensive (n=65)	Gestational hypertension (n=41)	Preeclampsia (n=24)	Significance of the difference between		
						Normotensive & Hypertensives	Normotensive & Preeclamptic	Hypertensive & Preeclamptic
Age (years)			29.4±3.3	29.4±4.3	28.5±4.6	0.894	0.586	0.575
Family history	HTN		3 (4.6%)	2 (4.9%)	4 (16.7%)	0.725	0.067	0.326
	PE		1 (1.5%)	2 (4.9%)	2 (8.3%)			
	GDM		3 (4.6 %)	1 (2.4%)	0			
	No		58 (89.3%)	36 (87.8%)	18 (75%)			
Gravidity	Primigravida		23 (35.4%)	19 (463%)	11 (45.8%)	0.261	0.368	0.968
	Multigravida		42 (64.6%)	22 (53.7%)	13 (54.2%)			
Parity	Nullipara		28 (43.1%)	23 (56.1%)	14 (58.3%)	0.191	0.201	0.861
	Multipara		37 (56.9%)	18 (43.9%)	10 (41.7%)			
BMI data	Weight (kg)		87.2±4.6	87.4±5.1	88.2±7	0.981	0.686	0.791
	Height (cm)		170±2	169.3±2.6	169.5±2.4	0.406	0.665	0.906
	BMI (kg/m^2)		30.2±1.7	30.5±1.8	30.7±2.7	0.762	0.462	0.878
	Grade	Overweight	27 (41.5%)	14 (34.1%)	6 (25%)	0.447	0.151	0.441
		Obese	40 (58.5%)	27 (65.9%)	18 (75%)			

Table (2): Systolic and diastolic blood pressure measures of the studied women during pregnancy

Data	Group	Normotensive (n=65)	Gestational hypertension (n=41)	Preeclampsia (n=24)	Significance of the difference between		
					Normotensive & Hypertensives	Normotensive & Preeclamptic	Hypertensive & Preeclamptic
SBP (mmHg)	12th	115.9±4.7	115±5.9	115.9±4.5	0.714	0.918	0.716
	16th	116.8±4.9	118±5.7	119±5.1	0.588	0.169	0.685
	20th	116.2±4.3	118.8±5	122.2±5.4	0.057	<0.001	0.008
	28th	117.8±4.4	121±5.5	132.8±17.2	0.249	<0.0001	<0.0001
	34th	118.1±5	123.4±6.2	132±7.4	0.0005	<0.0001	<0.0001
	38th	116±4.9	125±6	139.6±10.8	<0.0001	<0.0001	<0.0001
DBP (mmHg)	12th	76.7±4.2	76±5.4	77.5±4.5	0.880	0.779	0.482
	16th	77.5±3.9	77.1±4.8	79.2±5.2	0.833	0.278	0.606
	20th	77.9±4.5	78.7±4.5	81.3±4.5	0.725	0.005	0.043
	28th	78.4±3.7	79.9±39	87.5±9.8	0.429	<0.0001	<0.0001
	34th	79.6±3.7	86.2±2.3	92±5.7	<0.0001	<0.0001	<0.0001
	38th	81±3.5	88.1±2.3	101±8	<0.0001	<0.0001	<0.0001
Proteinuria (+1 on dipstick)	12th	0	0	0			
	16th	9 (13.8%)	13 (31.7%)	9 (37.5%)	0.027	0.014	0.634
	20th	11 (16.9%)	15 (36.6%)	14 (58.3%)	0.022	0.0001	0.089
	28th	22 (33.8%)	21 (51.2%)	24 (100%)	0.076	<0.0001	0.00004
	34th	36 (55.4%)	30 (73.2%)	24 (100%)	0.066	0.00007	0.0054
	38th	38 (58.5%)	33 (80.5%)	24 (100%)	0.019	0.0002	0.021

Table (3): The cytokines' serum levels estimated in samples of the studied women

Data Group	Normotensive (n=65)	Gestational hypertension (n=41)	Preeclampsia (n=24)	Significance of the difference between		
				Normotensive & Hypertensives	Normotensive & Preeclamptic	Hypertensive & Preeclamptic
TNF-α (ng/ml)	2.6±0.69	3.15±0.73	3.8±0.88	0.00009	<0.0001	0.0022
IL-10 (ng/ml)	30.4±8.7	22±5.72	16±4.73	<0.0001	<0.0001	0.0001
IL-6 (ng/ml)	14.5±5.56	31.4±11.61	38.7±12.59	<0.0001	<0.0001	0.0317
IL-10/IL-6 ratio	2.36±1	0.85±0.5	0.47±0.24	<0.0001	<0.0001	0.0035
PLGF (pg/ml)	324.6±131.8	179.6±74.1	137.4±46.7	<0.0001	<0.0001	0.0196

TNF-α: Tumor necrosis factor-α, IL: Interleukin; PLGF: Placental growth factor;

Table (4): Correlation analysis of at-enrolment data and the development of GHT, PE, and SBP measures obtained after the 20th GW of pregnancy

	Development of GHT		Development of PE		SBP at							
					20th GW		28th GW		34th GW		38th GW	
	Rho	p	Rho	p	Rho	p	Rho	p	Rho	p	Rho	p
Age	-0.52	0.560	-0.089	0.312	-0.101	0.255	-0.095	0.280	-0.123	0.162	-0.164	0.062
BMI	0.194	0.027	0.254	0.004	0.139	0.116	0.111	0.210	0.184	0.093	0.244	0.005
TNF-α	0.449	<0.001	0.409	<0.001	0.214	0.015	0.255	0.003	0.330	<0.001	0.449	<0.001
IL-6	0.758	<0.001	0.445	<0.001	0.252	0.004	0.252	0.004	0.362	<0.001	0.546	<0.001
IL-10	-0.582	<0.001	-0.483	<0.001	-0.258	0.003	-0.324	<0.001	-0.397	<0.001	-0.531	<0.001
IL-6/-10	-0.775	<0.001	-0.525	<0.001	-0.270	0.002	-0.308	<0.001	-0.419	<0.001	-0.602	<0.001
PLGF	-0.653	<0.001	-0.445	<0.001	-0.186	0.034	-0.231	0.008	-0.340	<0.001	0.703	<0.001

Table (5): Early predictors of altered blood pressure measures and the development of pregnancy-induced disorders

Variables	Prediction of gestational hypertension				Prediction of preeclampsia			
	Area under curve	Standard error	p	95% Confidence interval	Area under curve	Standard error	p	95% Confidence interval
Body mass index	0.612	0.050	0.028	0.515-0.709	0.689	0.068	0.004	0.556-0.822
High serum level of TNF-α	0.759	0.041	<0.001	0.678-0.840	0.804	0.052	<0.001	0.701-0.907
Low serum level of IL-10	0.166	0.034	<0.001	0.099-0.233	0.142	0.037	<0.001	0.070-0.215
High serum level of IL-6	0.937	0.019	<0.001	0.900-0.974	0.838	0.035	<0.001	0.769-0.907
IL-10/IL-6 ratio	0.052	0.018	<0.001	0.017-0.088	0.109	0.029	<0.001	0.053-0.165
Low serum levels of PLGF	0.123	0.029	<0.001	0.066-0.180	0.169	0.036	<0.001	0.098-0.240

TNF-α: Tumor necrosis factor-α; IL: Interleukin: PLGF: Placental growth factor

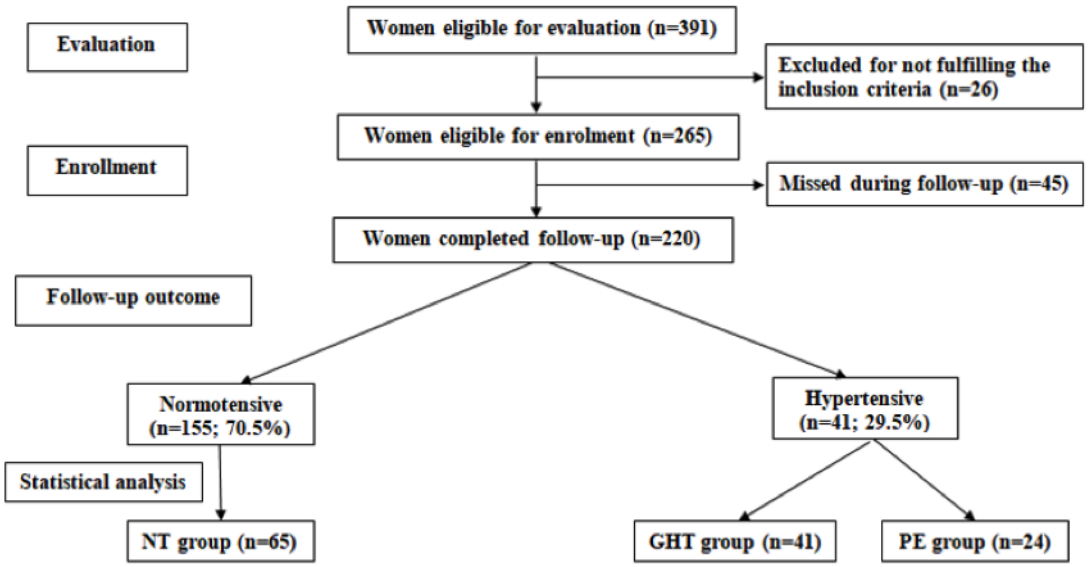


Figure 1: The flow-chart for enrolment and progress of the studied women

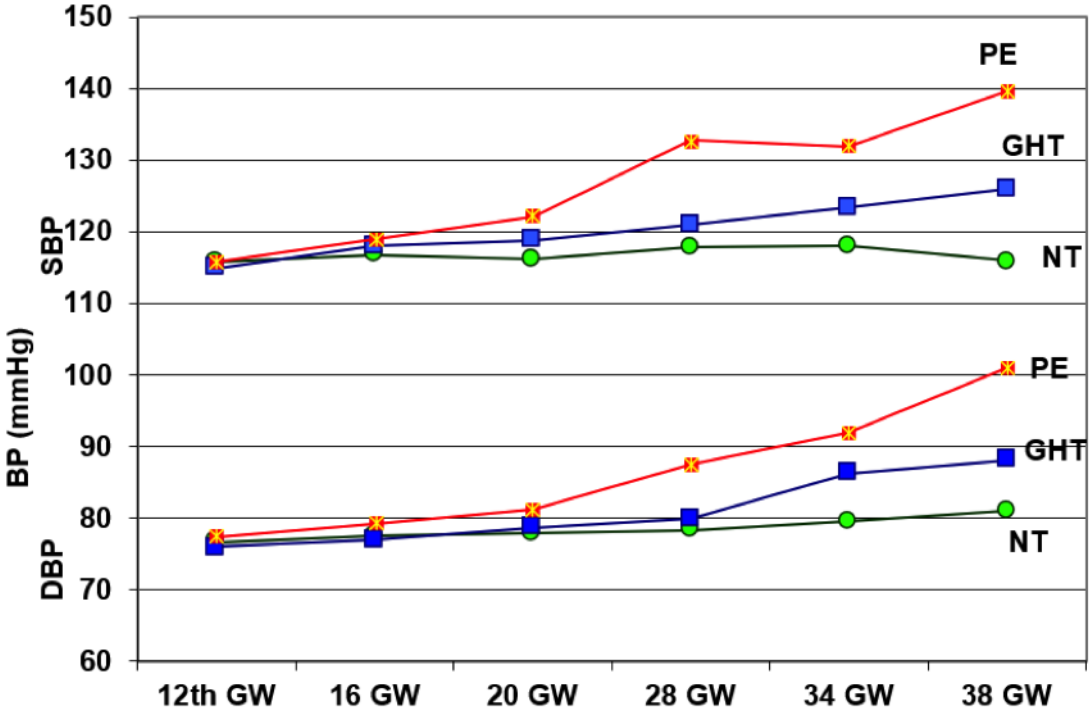


Figure 2 shows the progress of the measurements of systolic (SBP) and diastolic blood pressures (DBP) of the studied pregnant women throughout the follow-up period between the 12th and the 38th gestational weeks (GW).

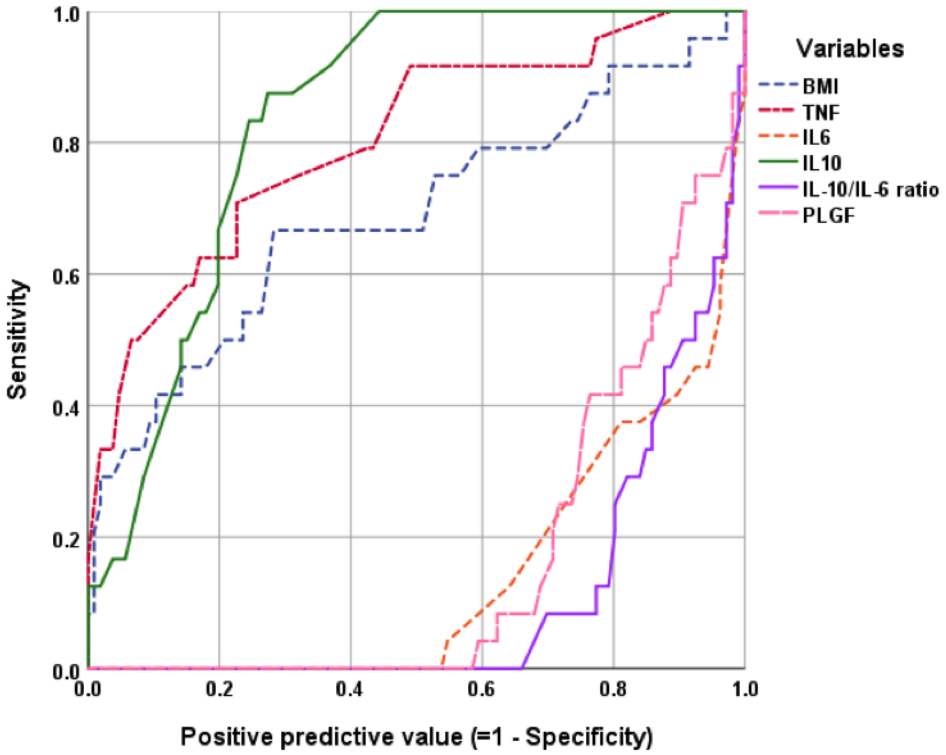
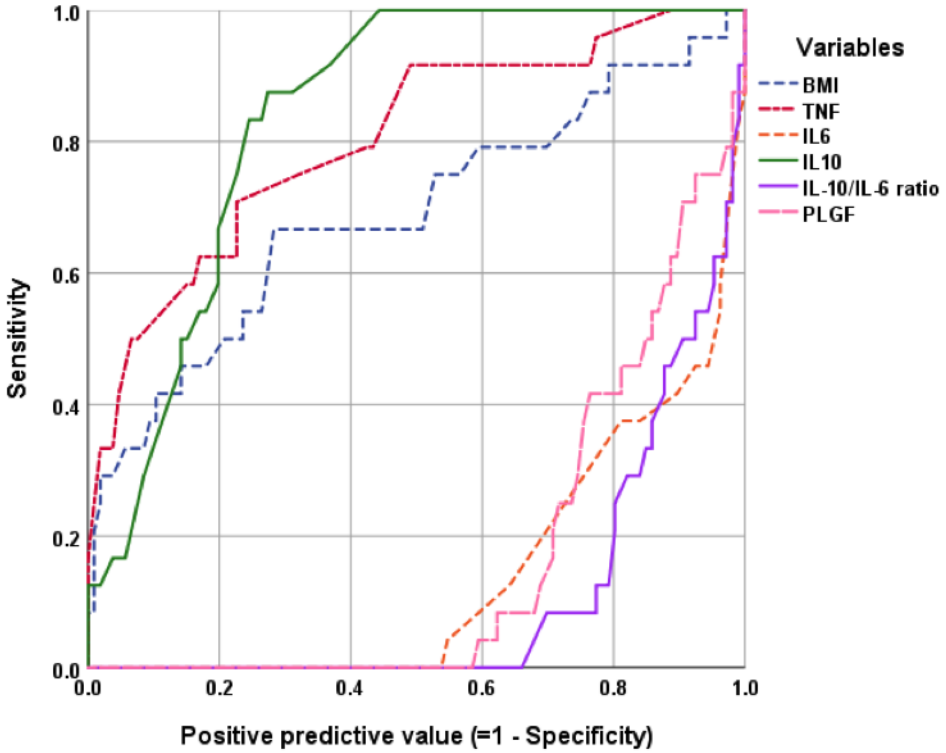


Figure 3 shows the AUCs for the data obtained at the time of patients' enrolment to be used to predict the possibility of having hypertensive disorders during pregnancy



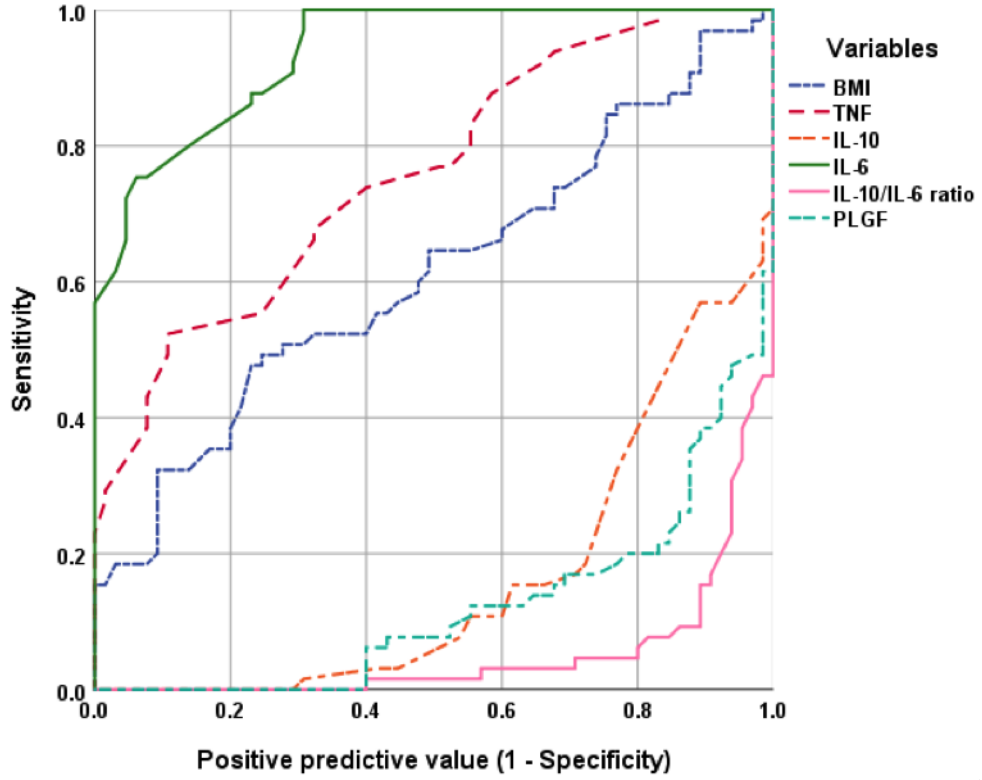


Figure 4 shows the areas under the ROC curve for the parameters determined at the time of enrolment of patients to elucidate their ability to be used as early predictors for the possibility of getting PE

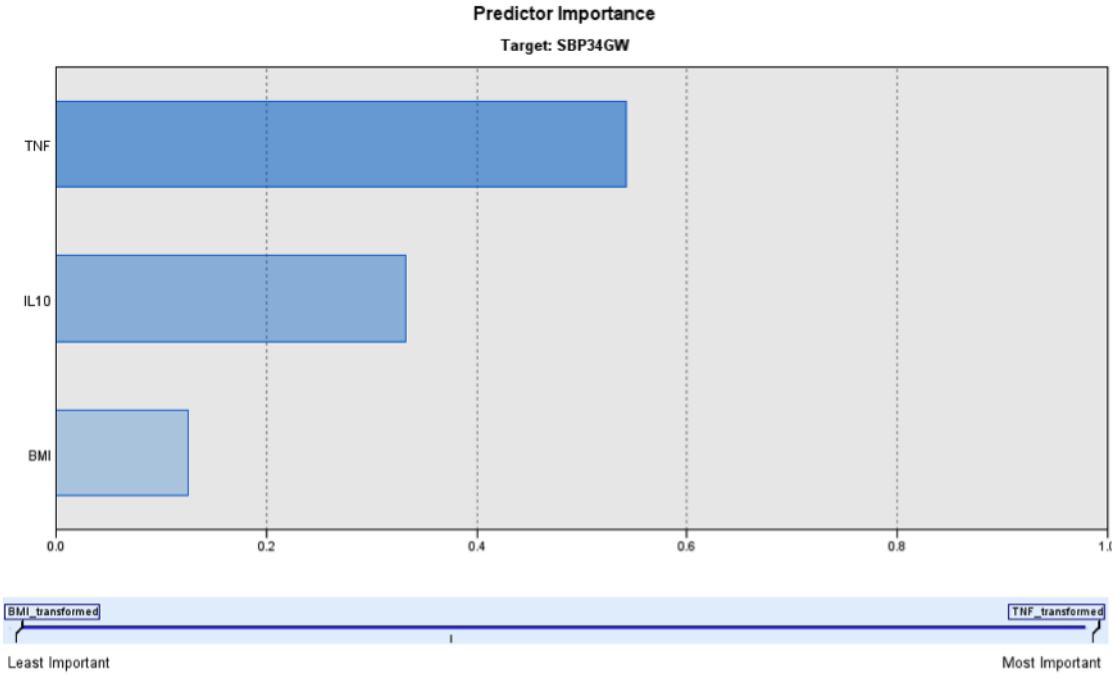


Figure 5 shows the percentages of the importance of the estimated cytokines' levels for the prediction of early PE as judged by the automatic linear model analysis