Estimation of IL-1β, Osteoprotegerin, and YKL-40: A Diagnostic Array for Prediction of Gestational Diabetes Mellitus complicated by Gestational Hypertension

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Keywords*:* Gestational insulin resistance (GIR), hypertension (GHT) and diabetes mellitus (GDM).

Abstract

Objectives: Estimation of serum levels of interleukin (IL)-1β, Osteoprotegerin (OPG) and YKL-40 early in pregnancy of normotensive normoglycemic women to find early predictors for development of gestational insulin resistance (GIR), hypertension (GHT) and/or diabetes mellitus (GDM).

Patients & Methods: The study included 255 pregnant women who were evaluated during their 1st antenatal visit (ANV-1) for age, body mass index (BMI), baseline systolic (SBP) and diastolic blood pressure (DBP) and gave blood samples for estimation of serum levels of insulin and the studied cytokines. Then, all women underwent 75-Oral glucose tolerance test (OGTT) for diagnosis of GDM and the homeostasis model assessment of IR (HOMA-IR) score at 6th and 24th GW.

Results: 32 women developed GIR that progressed to GDM, 38 women developed GHT, 13 developed GIR and GHT and 7 women developed the triad of GIR, GDM and GHT. There are significant differences in serum IL-1β, YKL-40 and OPG levels in women free and with these disorders. Serum levels of IL-1β showed positive, while serum OPG levels showed negative significant correlations with the incidence of pregnancy-induced disorders, while high serum YKL-40 was positively correlated with the incidence of GIR with GHT or GDM or both. ROC curve and Regression analyses defined combination of high DBP and serum YKL-40 as the significant early predictors for development of the three disorders, while high HOMA-IR score, SBP and serum IL-1β as the significant predictors for GHT, high BMI, DBP and serum YKL-40 as early predictors for GIR and GHT and high serum YKL-40 and low serum OPG levels as early predictors for GIR and DM.

Conclusion: Estimation of the studied array of cytokines at the 1st ANV has a high diagnostic value for the upcoming pregnancy-induced disorders single or combination with higher predictive value than the reliance on clinical data.

Keywords: Pregnancy, Insulin resistance, Diabetes mellitus, Hypertension, Cytokines, Early prediction

Introduction

Preeclampsia (PE) is a hypertensive disorder that develops during pregnancy and adversely affects both the mother and the fetus. PE is characterized by hypertension and proteinuria, and affects about 5% to 8% of pregnancies and causes maternal and perinatal mortality and morbidity (1). Maternal inflammatory and vascular endothelial dysfunction is important factors in the pathogenesis of PE (2).

Normally, during pregnancy, insulin resistance (IR) is increased as an adaptation process to enhance maternofetal nutrient transfer to meet the nutritional needs of the developing fetus, especially to glucose requirements (3). Increased proinflammatory cytokines during pregnancy are associated with hyperglycemia and IR and could be useful for predicting the development of gestational diabetes mellitus (GDM) (4).

Diabetes mellitus (DM) could be classified according to etiology and pathology as type 1, type 2 DM and GDM, which is characterized by hyperglycemia during pregnancy (5). Some studies have indicated that DM was related with inflammation (6).

Early studies suggested a reciprocal relationship between abnormal glucose metabolism and development of hypertension depending on the observation that individuals with abnormal glucose and insulin metabolism have higher incidence of hypertension, and patients with untreated essential hypertension have higher than normal plasma insulin concentrations and are resistant to insulin-stimulated glucose uptake (7). Recently, **Morikawa et al.** (8) found pregnant women with DM are at high risk for hypertensive disorder of pregnancy.

Hypothesis

The current study supposed a certain relation between development of GIR, DM and hypertension in pregnant women and these disorders may have a common pathogenic stack.

Design

Prospective comparative clinical trial

Setting

Benha University Hospitals in conjunction with some private centers

Objectives

Estimation of serum levels of interleukin (IL)-1β, Osteoprotegerin (OPG) and YKL-40 early in normotensive normoglycemic pregnant women in trial to find an early predictor for any of these pregnancy-induced disorders.

Patients & Methods

The study was conducted since June 2019 till Aug 2020 when the last enrolled case had reached her 36th gestational weeks (GW). The study protocol was approved by the Local Ethical Committee to include all women who attended the Antenatal (AN) Outpatient Clinics (OPC) at Benha University Hospitals for assurance of being pregnant, the 1st AN visit (ANV-1), were eligible for evaluation. At ANV-1, the collected demographic data included age, weight and height, and body mass index (BMI) was calculated in kg/m2 as weight (kg)/ height (m2) (9). The collected baseline obstetric and clinical data included number of previous pregnancies, deliveries and living children in multiparous women and medical history with special regard to essential hypertension, diabetes mellitus and kidney diseases.

Exclusion criteria include manifest DM, previous GDM in multipara women, morbid obesity with BMI>35 kg/m2 (10), essential hypertension, history of treatment for DM, hypertension, or other diseases treated by drugs well known to induce DM, hypertension or kidney affection, liver or renal diseases.

All pregnant women were asked to attend the OPC overnight fasting on the next day to the ANV-1 to give fasting blood samples for estimation of fasting blood glucose (FBG), fasting serum insulin (FSI), the study biomarkers, and to undergo the 75-Oral glucose tolerance test (OGTT) that entails estimation of FBG and postprandial blood glucose (PPBG) levels at one and two hours after taking a 75-gm oral glucose diet. Then, all women were allowed to relax while they were laying down and blood pressure was estimated on two occasions 4-hr apart and the median value of systolic and diastolic blood pressures (SBP & DBP) was determined and considered as the baseline measures. Only women with normal OGTT and median values of SBP and DBP and signed written fully informed consent, and free of exclusion criteria were enrolled in the study. All women were asked to attend the OPC at the 12th, 24th, 32nd, 36th GW for determination of SBP and DBP and at the 24th GW women must attend fasting to give blood samples for re-estimation of FSI and to repeat the 75-OGTT. Women who completed their pregnancy free of pregnancy induced disorders were grouped as control group; while those who developed these disorders were labelled according the type and multiplicity of disorders.

Diagnosis of Insulin resistance (IR) and gestational DM (GDM)

Insulin resistance (IR) was evaluated using the homeostasis model assessment of IR (HOMA-IR) score that was calculated according to the formula: fasting serum insulin (μU/ml) x [FBG (mg/ml)/18])/22.5; HOMA-IR score of >2 is considered abnormal (11). HOMA-IR score was determined twice at 6th and 24th GW. The results of the 75-OGTT were interpreted for diagnosis of GDM according to the recommendations of the International association of diabetes and pregnancy study groups (12) as follows: FBG ≥92 mg/dl, 1-h BG ≥180 mg/dl and 2-h BG ≥153 mg/dl indicate GDM.

Diagnosis and categorization of pre-eclampsia (PE)

Preeclampsia (PE) was defined according to the American Society of Hypertension (13) as development of gestational hypertension (GH) in a previously normotensive (NT) pregnant woman and is associated with proteinuria quantified as 1+ on dipstick. PE was categorized according to guidelines of American College of Obstetricians and Gynecologists as mild and severe according to BP measures obtained during follow-up visits, mild PE (MPE) was diagnosed if SBP and DBP were <160 and <110 mmHg, respectively with proteinuria of <2+ and absence of systemic manifestations. Severe PE (SPE) was diagnosed if elevated BP measures were associated with systemic manifestations or if SBP was ≥160 mmHg and DBP was ≥110 mmHg with proteinuria >2+ on a voided random urine sample (14). Concerning timing of development of PE in relation to gestational age, PE was considered of early-onset (EPE) if diagnosed prior to 34 GW and late (LPE) if diagnosed after the 34th GW (15, 16).

Investigations

Sampling: Venous blood samples (5 ml) were collected from the antecubital vein under complete aseptic conditions and were divided into two parts:

1. The first part was put in a tube containing sodium fluoride (2 mg sodium fluoride/ ml blood) to prevent glycolysis for estimation of blood glucose levels.
2. The second part was collected in plain tube, allowed to clot, centrifuged at 1500×g for 15 min and the serum samples were collected in clean dry Eppindorff tube to be stored at –70°C until assayed.

Estimated parameters

a. Blood glucose levels were estimated using glucose oxidase method (17).
b. Serum levels of IL-1β, OPG and YKL-40 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 insulin was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab200011, abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique (18).
    2. Human IL-1β was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab46052, abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique (19).
    3. Human OPG was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab189580, abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique (20).
    4. Human YKL-40 was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab255719, abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique (21).

Statistical analysis

Obtained data were presented as mean, standard deviation (SD), numbers and percentages. Results were analyzed using One-way ANOVA with post-hoc Tukey HSD Test and Chi-square test (X2 test). Possible relationships were investigated using Spearman's linear regression for non-parametric data. Sensitivity & specificity of estimated parameters as predictors for PREGNANCY-INDUCED DISORDERS 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 and then were verified using the Regression analysis, Stepwise method. Statistical analysis was conducted using the IBM SPSS (Version 23, 2015) for Windows statistical package. P value <0.05 was considered statistically significant.

Results

The study included 268 pregnant women eligible for evaluation, 13 women were excluded and 255 women were included in the study (Fig. 1). At the end of the study duration 87 women developed pregnancy-induced disease, while the remaining 168 women completed their pregnancy free (Control group). Sixty-two women (24.3%) developed GIR with HOMA-IR score of ≥2, unfortunately, 32 women (12.5%) of those had GIR gave positive OGTT and were considered GDM. Thirty-eight women developed GHT, 16 developed early PE and 22 had late PE; 10 women had severe PE and 28 had late PE. Seven women had GIR, GDM and GHT, while 13 women had GIR and GHT and 18 women had GHT without GIR or GDM. At ANV-1, women who developed pregnancy-induced diseases showed significant differences

in comparison to those completed their pregnancy free of diseases concerning enrolment data as shown in table 1.

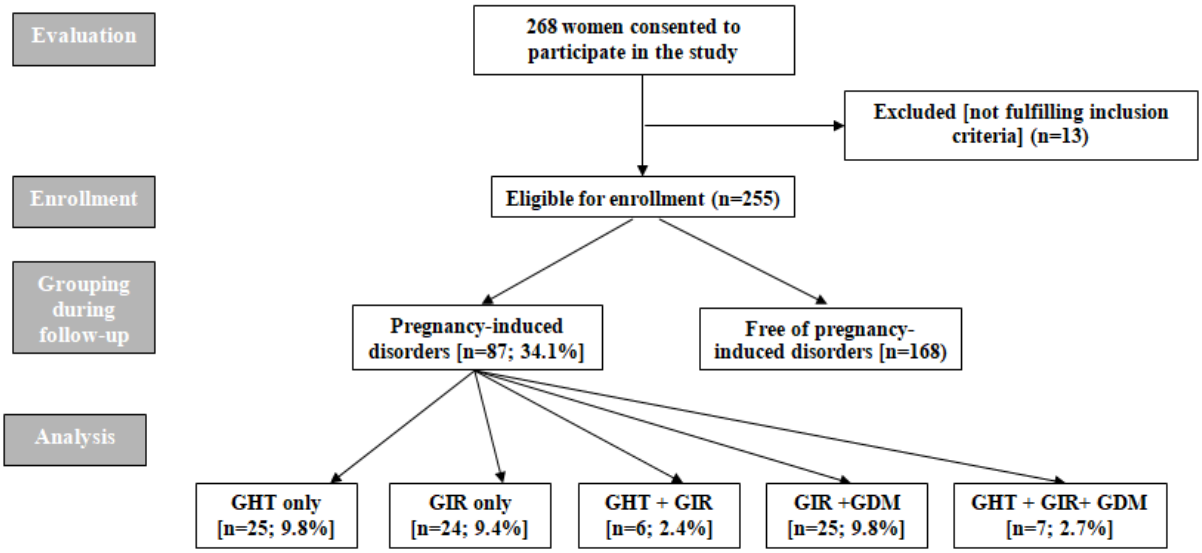

Figure 1: Consort Flow sheet

Table (1): Enrolment demographic and clinical data of studied women

Data		Control group (n=168)	Gestational diseases						P value
			GHT only	GIR only	GHT +IR	GDM+ GIR	GHT+ IR+GDM	Total	
Number (%)		168 (68.6%)	25 (9.8%)	24 (9.4%)	6 (2.4%)	25 (9.8%)	7 (2.7%)	87 (34.1%)	
Age (years)		28.3±2.5	28.7±2.3	26.6±2.9	28±3.9	28.1±3.2	27.7±4.2	27.5±3	0.022
BMI (kg/m^2)		28.2±1.7	29.1±0.8	28.4±1.9	31±2.6	28±2.6	28.1±3.9	28.8±2	0.009
Obstetric history	Primigravida	56 (33.3%)	7 (28%)	24 (100%)	6 (100%)	11 (44%)	6 (85.7%)	54 (62.1%)	0.000022
	Primipara	60 (35.7%)	8 (32%)	24 (100%)	6 (100%)	11 (44%)	6 (85.7%)	55 (63.2%)	0.000029
75-OGTT	FBG (mg/dl)	80.3±5.8	81.5±6.8	80.8±4.2	81.2±2.3	79.9±7.9	78.1±11.2	80.7±5.6	0.615
	PPBG (mg/dl)	118.1±6.8	119.6±4.8	120.6±4.5	121.5±4.2	117.6±10.6	115.9±16.2	119.8±5.5	0.045
HOMA-IR	FSI (µU/ml)	3.65±0.86	3.66±0.98	5.84±0.84	6.23±0.63	4.16±1.66	4.4±1.97	5.9±1.94	<0.0001
	HOMA score	0.74±0.18	0.75±0.21	1.18±0.17	1.27±0.15	0.84±0.34	0.87±0.4	1.2±0.39	<0.0001
Blood pressure	SBP (mmHg)	104.5±5.9	117.6±9	104.1±5.5	124±6	104.4±9.4	103.5±14.8	111.9±9.8	<0.0001
	DBP (mmHg)	68.8±2.4	74.7±5.2	69.8±2.8	79.5±4.9	68.6±5.4	68.3±9.4	72.7±5.3	<0.0001

Data are presented as mean; standard division (SD), numbers & percentages; BMI: Body mass index; 75-OGTT: 75-oral glucose tolerance test; FBG: Fasting blood glucose; PPBG: Postprandial blood glucose; FSI: Fasting serum insulin; HOMA-IR: Homeostasis model assessment of insulin resistance; SBP: systolic blood pressure; DBP: Diastolic blood pressure; GHT: Gestational hypertension; IR: Gestational insulin resistance; GDM: Gestational diabetes mellitus; P value indicates significance of difference between free and diseased group; P<0.05: indicates significant difference

At the 24th GW, women of control group had significantly lower FBG in comparison to women who developed GIR and GDM with or without GHT, while the differences were non-significant versus FBG of women who developed GHT or GIR only. Women who developed only GHT or GIR had significantly lower FBG levels compared to women who developed GIR and GDM with or without GHT. As regards PPBG levels estimated at the 24th GW, women of control group had non-significantly lower levels than women who developed GHT only, while was significantly lower than levels estimated in all women of other groups. Women developed GHT only had significantly lower PPBG level in comparison to women who developed GHT and GIR with or without GDM. Estimated FSI and calculated HOMA scores were significantly lower in women of control group and women who developed GHT in comparison to women of other groups (Table 2).

Table (2): Results of 75-OGTT and HOMA-IR score calculation at the 24th GW studied women categorized according to the diagnosed pregnancy-induced disorder

Group Variables		Control group (n=168)	GHT only (n=25)	GIR only (n=24)	GHT +GIR (n=6)	GDM+ GIR (n=25)	GHT+IR+ GDM (n=7)
75-OGTT	FBG (mg/dl)	86.4±6	88.2±7.4	87.2±2.9	88±2	110.2±9	109.6±10.9
	P1		0.179	0.539	0.518	<0.0001	<0.0001
	P2				0.949		<0.0001
	P3				0.509	<0.0001	<0.0001
	PPBG (mg/dl)	127.7±9.4	129.8±9.8	140.5±6.6	142.7±8.5	190.4±17.5	211.6±17.8
	P1		0.295	<0.0001	0.0002	<0.0001	<0.0001
	P2				0.0065		<0.0001
	P3				0.494	<0.0001	<0.0001
HOMA-IR	FSI (µU/ml)	5.21±0.85	5.39±0.92	9.55±0.5	10.15±0.8	10.4±1.29	11.4±1.39
	P1		0.327	<0.0001	<0.0001	<0.0001	<0.0001
	P2				<0.0001		<0.0001
	P3				0.0278	<0.0001	<0.0001
	HOMA score	1.13±0.2	1.19±0.25	2.11±0.11	2.24±0.15	2.87±0.42	3.1±0.27
	P1		0.142	<0.0001	0.0002	<0.0001	<0.0001
	P2				<0.0001		<0.0001
	P3				0.248	<0.0001	<0.0001

Data are presented as mean; standard division (SD); 75-OGTT: 75-oral glucose tolerance test; FBG: Fasting blood glucose; PPBG: Postprandial blood glucose; FSI: Fasting serum insulin; HOMA-IR: Homeostasis model assessment of insulin resistance; GHT: Gestational hypertension; IR: Gestational insulin resistance; GDM: Gestational diabetes mellitus; P1 indicates significance of difference versus women free of pregnancy induced diseases; P2 indicates significance of difference versus women developed GHT only; P2 indicates significance of difference versus women developed GIR only; P<0.05: indicates significant difference

Estimated blood pressure measures in patients who developed GHT alone or with GIR and/or GDM throughout the study duration were significantly higher in comparison to measures estimated in control women, or developed GIR with or without GDM. Moreover, estimated blood pressure measures during pregnancy course were significantly higher in women developed GHT and GIR with or without GDM in comparison to measures of women who developed IR only. Women who developed GIR and GDM had significantly higher blood pressure measures at the 12th GW in comparison to women who completed their pregnancy of control group, but the differences became non-significant thereafter. Moreover, the differences in blood pressure measures between women who developed GIR only versus those who developed GIR and GDM were non-significant (Table 3).

Table (3): Blood pressure measures during pregnancy course in studied women categorized according the associated pregnancy-induced disorders

	Control group (n=168)		GHT (n=25)		GIR only (n=24)		GHT +GIR (n=6)		GDM+ GIR (n=25)		GHT+GIR+ GDM (n=7)	
	SBP	DBP	SBP	DBP	SBP	DBP	SBP	DBP	SBP	DBP	SBP	DBP
ANV	105±6	69±2	118±9	75±5	104±6	70±3	124±6	79±5	104±9	69±5	104±15	68±9
12th	108±5	71±2	124±8	80±5	107±5	73±2	131±8	85±6	111±7	72±3	128±5	85±4
P1			<0.0001	<0.0001	0.616	0.018	<0.0001	<0.0001	0.027	0.037	<0.0001	<0.0001
P2							0.064	0.091			0.272	0.028
P3							<0.0001	<0.0001	0.072	0.498	<0.0001	<0.0001
24th	112±5	74±3	138±18	91±14	112±5	76±3	147±19	98±16	114±7	75±3	142±15	94±11
P1			<0.0001	<0.0001	0.735	0.008	<0.0001	<0.0001	0.107	0.197	<0.0001	<0.0001
P2							0.278	0.269			0.648	0.566
P3							<0.0001	<0.0001	0.201	0.342	<0.0001	<0.0001
32nd	116±5	77±3	133±7	87±4	115±4.9	78±3	140±3	90±4	117±6	78±3	136±4.8	90±3
P1			<0.0001	<0.0001	0.169	0.387	<0.0001	<0.0001	0.391	0.112	<0.0001	<0.0001
P2							0.067	0.194			0.528	0.124
P3							<0.0001	<0.0001	0.125	0.539	<0.0001	<0.0001
36th	120±5	81±3	146±10	95±9	120±3.8	81±3	145±9	96±6	120±5	82±3	144±14	94±8
P1			<0.0001	<0.0001	0.921	0.492	<0.0001	<0.0001	0.825	0.247	<0.0001	<0.0001
P2							0.863	0.688			0.699	0.842
P3							<0.0001	<0.0001	0.925	0.685	<0.0001	<0.0001

Data are presented as mean; standard division (SD); ANV: First antenatal visit; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; GHT: Gestational hypertension; IR: Gestational insulin resistance; GDM: Gestational diabetes mellitus; P1 indicates significance of difference versus women free of pregnancy induced diseases; P2 indicates significance of difference versus women developed GHT only; P2 indicates significance of difference versus women developed GIR only; P<0.05: indicates significant difference

Mean serum levels of IL-1β and YKL-40 were significantly lower, while serum OPG levels were significantly higher in control women in comparison to women who developed pregnancy-induced disorders. Also, women who develop GIR had significantly higher serum lower IL-1β and YKL-40 levels, while had significantly lower serum OPG levels in comparison to those developed GIR in addition to GHT and/or GDM. Serum IL-1β showed non-significant differences between women who developed GHT only or with GIR and GDM. On contrary, serum levels of OPG were significantly higher and serum YKL-40 were significantly lower in women developed GHT only in comparison to women who developed additional disorders (Table 4).

Table (4): Serum levels of IL-1β, OPG and YKL-40 estimated at time of enrolment of studied women

Group Parameters		Control group (n=168)	GHT only (n=25)	GIR only (n=24)	GHT +GIR (n=6)	GDM+ GIR (n=25)	GHT+GIR+ GDM (n=7)
IL-1β (ng/ml)	Level	45.7±7.12	139.3±11.9	115.2±17.2	138.5±6.7	118.2±19.1	143.1±19.3
	P1		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	P2				0.979		0.514
	P3				0.0032	0.567	0.0009
OPG	Level	2.21±0.09	1.95±0.07	1.94±0.04	1.85±0.07	1.85±0.11	1.82±0.1
	P1		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	P2				0.0055		0.0006
	P3				0.0005	0.00048	0.00008
YKL-40	Level	36.79±11.2	49.34±9.5	67.2±10.8	82.45±3.76	75.1±5	88.6±4.5
	P1		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	P2				<0.0001		<0.0001
	P3				0.0022	0.0018	0.00002

Data are presented as mean; standard division (SD); IL-1β: Interleukin-1β; OPG: Osteoprotegren; GHT: Gestational hypertension; IR: Gestational insulin resistance; GDM: Gestational diabetes mellitus; P1 indicates significance of difference versus women free of pregnancy induced diseases; P2 indicates significance of difference versus women developed GHT only; P2 indicates significance of difference versus women developed GIR only; P<0.05: indicates significant difference

Spearman' correlation of demographic, clinical and laboratory data obtained at ANV-1 for the possibility of development pregnancy-induced disorders showed significant correlation between maternal age and previous gravidity, HOMA-IR score, blood pressure measures and serum levels of studied parameters and the possibility of development of GHT, IR and DM. While there was significant correlation between BMI, previous gravidity and parity, HOMA-IR score and studied parameters and the possibility of development of GIR with HT or DM (Table 5).

Table (5): Spearman's correlation coefficient for the possibility of development of pregnancy-induced disorders and demographic, clinical and laboratory data determined at ANV-1 of studied women

Pregnancy-induced disorders / Variable	Possibility of development of GHT+GIR+GDM		Possibility of development of GHT		Possibility of development of GIR+GHT		Possibility of development of GIR+GDM	
	Rho	p	Rho	p	Rho	p	Rho	p
Age	-0.153	0.014	0.039	0.540	0.010	0.872	-0.028	0.659
BMI	0.114	0.069	0.067	0.495	0.166	0.008	0.156	0.013
Gravidity	-0.121	0.047	-0.265	0.039	-0.164	0.009	0.039	0.688
Parity	-0.115	0.068	0.048	0.443	-0.159	0.011	0.038	0.698
FBG	-0.037	0.554	0.080	0.203	0.076	0.229	-0.102	0.553
2-hr PPBG	-0.016	0.799	0.052	0.412	0.008	0.893	-0.054	0.387
HOMA-IR score	0.247	<0.001	-0.123	0.049	0.184	0.003	0.473	<0.001
SBP	0.238	<0.001	0.369	<0.001	0.233	<0.001	0.068	0.280
DBP	0.241	<0.001	0.300	<0.001	0.218	<0.001	0.025	0.693
Serum IL-1β	0.239	<0.001	0.449	<0.001	0.214	0.001	0.326	<0.001
Serum OPG	-0.237	<0.001	-0.302	<0.001	-0.208	0.001	-0.440	<0.001
Serum YKL-40	0.281	<0.001	0.113	0.073	0.242	<0.001	0.427	<0.001

ANV: First antenatal visit; BMI: Body mass index; FBG: Fasting blood glucose; PPBG: Postprandial blood glucose; HOMA-IR: Homeostasis model assessment of insulin resistance; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; IL-1β: Interleukin-1β; OPG: Osteoprotegren; GHT: Gestational hypertension; IR: Gestational insulin resistance; GDM: Gestational diabetes mellitus; P indicates the significance of the correlation; -: indicates negative correlation

For prediction of the possibility of development of the triad of GHT, IR and DM, the correlated variables were studied using the ROC curve analysis which

excluded the gravidity as an early predictor (Fig. 2); then the Regression analysis for verification of variables with significant AUC on ROC analysis defined combination of high DBP and serum YKL-40 are the significant early predictors for the development of the three disorders.

On the other hand, the ROC curve analysis excluded the gravidity as an early predictor for the development of GHT alone (Fig. 3); but Regression analysis defined combination of high HOMA-IR score, SBP and serum IL-1β as the significant early predictors for the development of GHT.

Regarding the possibility of development of GIR and HT, ROC curve analysis assured the early predictability of the variables that showed positive correlation with the development of these two disorders (Fig. 4), Regression analysis defined high BMI, DBP and serum YKL-40 as the early significant predictor for development of combination of GIR and HT. Concerning the early prediction of GIR and DM, ROC curve analysis excluded BMI and DBP as early predictors (Fig. 5) and Regression analysis defined high serum YKL-40 and low serum OPG levels as the early predictors for GIR and DM.

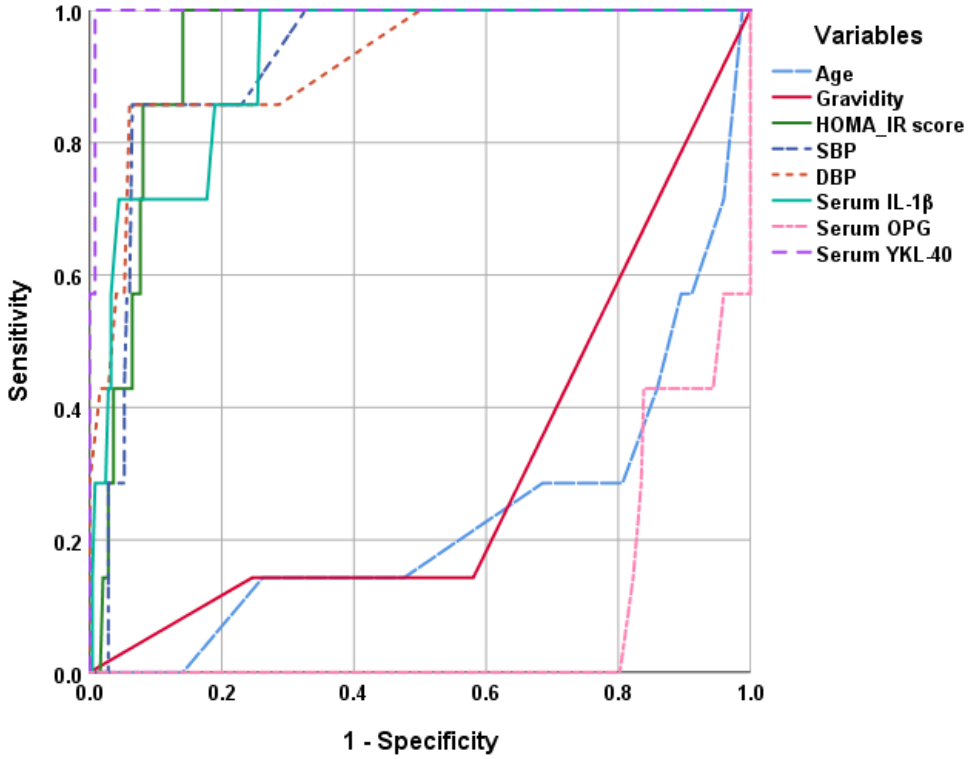

Fig. (2): ROC curve analysis for demographic, clinical and laboratory data determined at ANV-1 as predictors for development of the triad of GHT, GIR and GDM during pregnancy

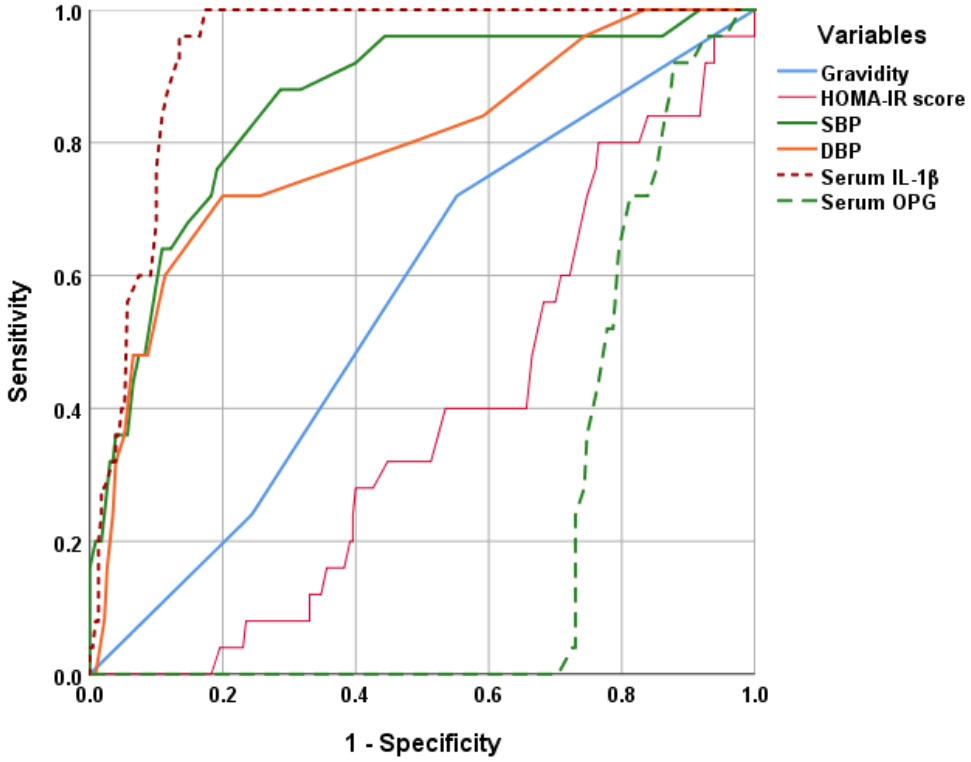

Fig. (3): ROC curve analysis for demographic, clinical and laboratory data determined at ANV-1 as predictors for development of GHT during pregnancy

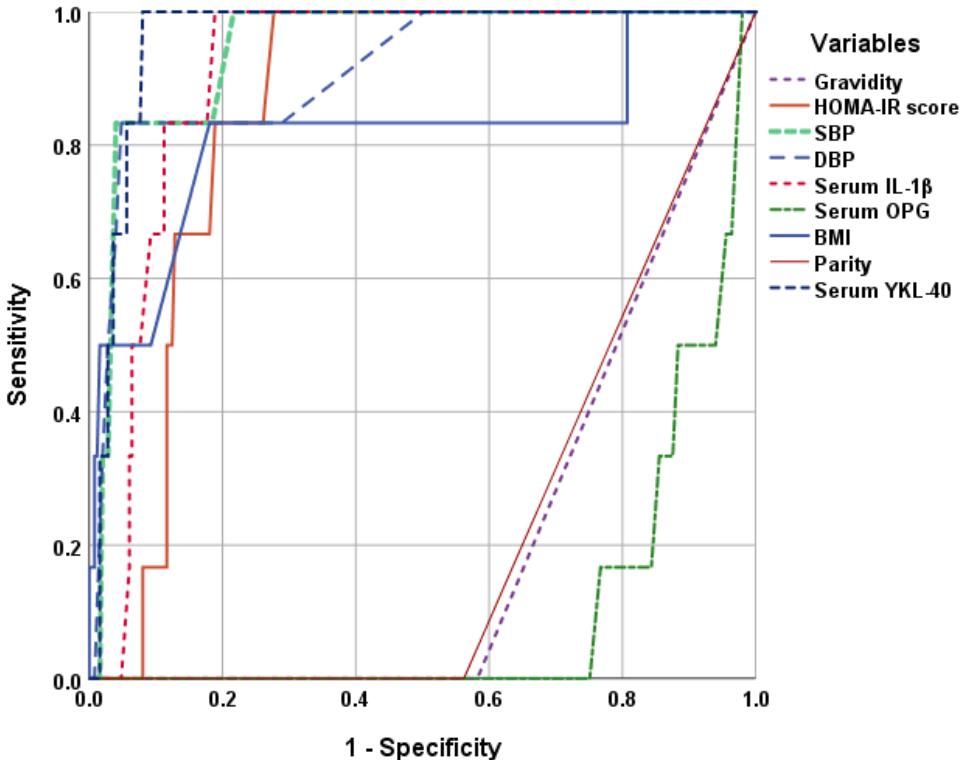

Fig. (4): ROC curve analysis for demographic, clinical and laboratory data determined at ANV-1 as predictors for the triad of GHT, GIR and GDM

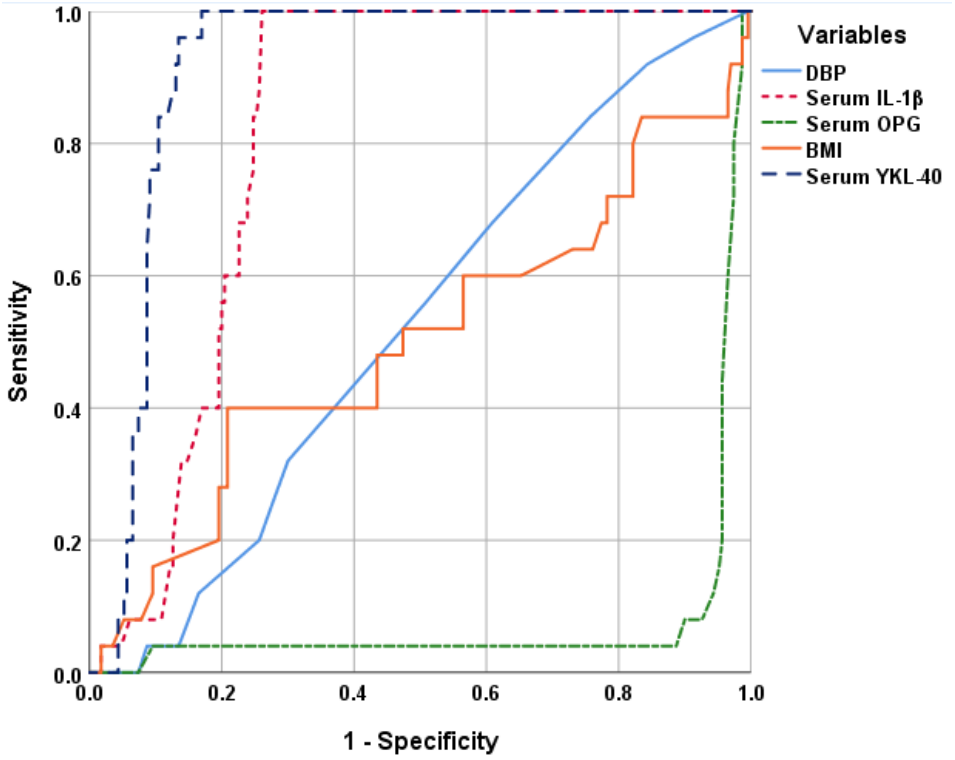

Fig. (5): ROC curve analysis for demographic, clinical and laboratory data determined at ANV-1 as predictors for development of GIR and GDM during pregnancy

Discussion

Pregnancy-induced disorders were detected in 87 pregnant women for a frequency of 31.4% of the studied population, 7 women (2.7%) had developed the triad of GIR, GDM and GHT, 31 women developed GIR with GHT or GDM. These figures point to a certain pathogenic mechanism underlying the development of these disorders alone or together and may suggest a reciprocal relation between the three disorders.

Multiple recent studied tried to explain the coincidence of this triad of disorders; **Luo et al.** (22) experimentally indicated that reduced dopaminergic neuronal activity at the area communicating with the hypothalamic suprachiasmatic nuclei contributes significantly to increased sympathetic tone with subsequent development of metabolic syndrome and hypertension. Clinically, **Motta et al.** (23) found acute physical and mental stress is associated with significant increase in blood levels of free fatty acids and norepinephrine with elevation of DBP and increased peripheral vascular resistance of the large and small arteries in comparison to baseline and attributed pathological increase of blood pressure and metabolic changes to sustained increase in sympathetic activity. These explanations for coincidence of hypertension and metabolic syndrome could be a possible mechanism for that occurring during pregnancy which is documented as a state of continued physical and mental stress with an anxious or traumatic experience for some women that is closely associated with their psychological well-being (24) and is associated with several mood changes (25). As another explanation, **Suárez-Cuenca et al.** (26) attributed the coincidence of this triad to the effect of obesity and increased weight gain with subsequent change of adipocyte volume and suggested that hypertension-resistin-HbA1c interactions were associated with larger subcutaneous adipocytes; while potential insulin-adiponectin associations were observed for larger visceral adipocytes

and thus suggested a relation between adipocyte morphology and source with cardiometabolic and atherogenic risk in population with obesity

These attributions could explain and support the reported data where the incidence of the studied disorders showed positive significant correlation with BMI and HOMA-IR scores that were determined at the 1st antenatal visit (ANV-1). Also, systolic and diastolic blood pressure measures determined at ANV-1 were positively correlated with BMI and with the oncoming GHT alone or with GIR.

The current study also reported significant differences in serum IL-1β, YKL-40 and OPG levels in women free and with PDI and serum levels of IL-1β showed positive, while serum OPG levels showed negative significant correlations with the incidence of PDI, irrespective of being single, double or triple disorders, while high serum YKL-40 was positively correlated with the incidence of GIR with GHT or with GDM or with both. These findings indicated a relationship between altered levels of these cytokines and the development and/or severity of disorders, irrespective of being a causal or resultant relation.

The obtained data go in hand with and support the results of previous studies evaluated the levels of these cytokines in pregnant women, despite being studied separately; wherein **Nunes et al.** (27) reported higher plasma concentrations of IL-1β, TGF-β1, and TNF-α in women with severe than mild PE, and in comparison to normotensive pregnant women, and all PE women showed decreased plasma levels of sCD163 and IL-10 and concluded that in PE women there is an impairment in the modulation of the systemic inflammatory response. Thereafter, **Emara et al.** (6) found serum level of inflammatory markers were significantly higher in diabetic patients than controls with a significant positive correlation between with FBG, serum creatinine, total cholesterol, LDL-C, HbA1c, and microalbumin/creatinine ratio. Also, **Lin et al.** (28) using PE-induced rat model found administration of E2 decreased inflammation, NO levels and altered the uterine angiogenic status and significantly suppressed the toll-like receptor 4 signaling pathway with attenuation of high BP, fetal weight, proteinuria, inflammatory response, oxidative stress and endothelial dysfunction and thus attributed these disturbances with subsequent development of PE to low maternal E2 levels.

Recently, **Luo et al.** (29) demonstrated that serum YKL-40 levels are increased in diabetics and are positively associated with the severe degree of albuminuria and suggested that YKL-40 could be a marker along with other inflammatory markers, if DM is suspected. Also, **Huang et al.** (30) experimentally detected glucose intolerance, decreased β-cell proliferation and serum insulin levels in placenta-specific OPG knockdown pregnant rat model and suggested that placenta-derived OPG regulated glucose homeostasis during pregnancy via enhancement of β-cell proliferation and may be used as a potential therapy for GDM.

Conclusion

Pregnancy is a stressful condition and is associated with coincidence of pregnancy-induced disorders in large number of women and this may endanger both the mother and the fetus. Disturbed immune milieu in direction of inflammation, progressive increase of BMI with subsequent release of adipocytokines may be a common stack for development of pregnancy-induced disorders, so coincidence of more than one disorder is not infrequent event. Estimation of the studied array of cytokines at the 1st antenatal visit has a high diagnostic value for the upcoming pregnancy-induced disorders either single or combination. Predictive value of these

cytokines was superior to the reliance on clinical data especially in communities where follow-up may be infrequent.

Limitations

The study is limited to one center serving certain locality, so multicenter wider-scale studies are advocated to evaluate the coincidence of multiple pregnancy-induced disorders and the diagnostic validity of the supposed diagnostic array.

Recommendation

Prophylactic physical training and interventions to reduce weight gain must be tried as a trial to reduce the incidence and severity of pregnancy-induced disorders.

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