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RESEARCH ARTICLE

ESTIMATION OF SERUM GLYCODELIN A, INSULIN-LIKE GROWTH FACTOR-1 AND INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-3 AT 4TH WEEK GESTATIONAL AGE AS PREDICTORS FOR EARLY PREGNANCY LOSS IN PREGNANT WOMEN WITH POLYCYSTIC OVARY SYNDROME.

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Abstract

Objectives: To evaluate predictability of estimation of serum levels of glycodelin A (GdA), insulin growth factor-1 (IGF-1) and its binding protein-3 (IGFBP-3) at the 4th week gestation age (GA) for development of early pregnancy loss (EPL) in primigravida with polycystic ovary syndrome (PCOS).

Patients & Methods: The study included 45 primigravida PCOS women developed EPL (Study group) and 45 primigravida PCOS women completed the observation period uneventfully (Control group). At the 4th wk GA, all women underwent clinical examination and pregnancy was assured, then all women gave fasting blood samples for estimation of fasting blood glucose (FBG), serum insulin, GdA, IGF-1 and IGFBP-3. Insulin resistance (IR) was evaluated using the homeostasis model assessment IR (HOMA-IR) score.

Results: Study women had significantly higher FBG and HOMA-IR score than control women. Estimated 4-wk GA serum levels of IGF-1 were significantly higher, while serum IGFBP-3 and GdA were significantly lower in study versus control PCOS women. Development of EPL showed positive significant correlation with high FBG, HOMA-IR score and high serum IGF-1 levels, while showed negative significant correlation with serum IGFBP-3 and GdA levels. Statistical analyses defined high serum IGF-1 as significant sensitive predictor, while low serum glycodelin A, IGFBP-1 and FBG as specific predictors for development of EPL, in decreasing order of significance. Conclusion: Low serum GdA and IGFBP-3 levels and high IGF-1 levels estimated at the 4th wk GA may underlie the development of EPL in pregnant PCOS women. Estimation of three markers as multiple marker panels could predict EPL with high sensitivity and specificity.

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Introduction:-

Polycystic ovary syndrome (PCOS) is a common endocrine disorder that has profound implications for women throughout their reproductive life ⁽¹⁾. PCOS affects 6 to 15% of reproductive age women worldwide ⁽²⁾ and is associated with increased risk of pregnancy-related complications ⁽¹⁾ that may possibly explained by presence of several endometrial abnormalities ⁽³⁾.

The pathophysiology of PCOS is very unique, and several hormonal and metabolic changes occur ⁽⁴⁾. Insulin resistance (IR) in PCOS women may be related to lower expression of GLUT4 ⁽⁵⁾ and/or a disturbance in the coupling of the stimulation of insulin receptor by insulin ⁽⁶⁾ ending at compensatory increase in insulin concentration despite of increased blood glucose levels ⁽⁵⁾.

Close relationships have been demonstrated between IR and serum insulin-related growth factor (IGF)-1 level in patient of PCOS ⁽⁴⁾. The efficacy of IGF-1 depends on the expression and availability of a family of six types of IGF-binding proteins (IGFBP) ⁽⁷⁾. In humans, IGFBP-3 is the most abundant representing >80% of IGFBP, has great affinity for IGF-1 and -2 and is responsible for the maintenance of the circulating IGF-1 levels ⁽⁸⁾. IGFBP-3 alone or in conjunction with other adipokines, is also associated with IR ⁽⁹⁾.

Human glycodelin (Gd) is an abundant glycoprotein and is a potential paracrine regulator involved in crucial biological processes such as reproduction and immune reaction ⁽¹⁰⁾. Glycodelin contains four isoforms with diverse biological functions. Glycodelin-A (GdA) is potentially a diagnostic marker for receptivity marker of the secretory endometrium ⁽¹¹⁾.

Objectives:-

This study aimed to evaluate the predictability of estimation of serum levels of GdA, IGF-1 and IGFBP-3 at the 4th week of gestation for development of early pregnancy loss (EPL) in pregnant women with PCOS.

Design:-

Prospective selective comparative clinical trial

Setting:-

Benha University Hospitals

Patients & Methods:-

The study protocol was approved by the Local Ethical Committee and all enrolled women signed written fully informed consent. All primigravida with PCOS between 4th and 5th wk GA, accepting to attend the OPC 4-weekly thereafter unless if there was any indication to attend in-between regular visits, accepting to give a phone number for follow-up if can't attend the OPC were included in the study. The study targets to collect > 40 primigravida with PCOS who developed pregnancy loss within the 1st 12-wk gestation period as study group and then a similar number of primigravida PCOS women who continued their pregnancy successfully for >12 wks as control group. Exclusion criteria included uterine anatomical anomalies, BMI>35 kg/m², endocrinopathy, multiple pregnancy endometriosis were excluded.

Diagnosis of pregnancy relied on positive serum pregnancy test and was assured using TVU. Gestational age (GA) was defined as the number of weeks since the last menstrual period. All women had complete history taking and full clinical examination including body height and weight, and body mass index (BMI) was calculated as weight (kg)/height (m²) ⁽¹²⁾. Obesity grades were defined after the WHO expert consultation ⁽¹³⁾ as BMI <24.9 as average, 25-<30 kg/m² as overweight, BMI ≥30-<35 kg/m² as obese and BMI ≥35 kg/m² as morbid obese. Insulin resistance (IR) was measured by homeostasis model assessment IR (HOMA-IR) score. The HOMA-IR score was calculated as (fasting serum insulin (μU/ml) x [fasting plasma glucose (mg/ml)/18])/22.5 ⁽¹⁴⁾ considering an abnormal HOMA-index >2 ⁽¹⁵⁾.

Laboratory investigations:-

A fasting venous blood sample (5 ml) was obtained under complete aseptic conditions from the antecubital vein at the 1st visit to OPC and divided into two parts:

1. The first was kept in a plane container and was left to clot then serum separated by centrifugation at 3000 rpm for 5 min. and stored at -80°C .
2. The 2nd part was collected in an EDTA containing tube for estimation of fasting blood glucose (FBG) level

Investigations:-

1. Blood glucose levels were estimated using glucose oxidase method ⁽¹⁶⁾.
2. Estimations of plasma insulin concentrations were determined using a commercial ELISA kit (Mercodia ELISA; ALPCO Diagnostics, Uppsala, Sweden) ⁽¹⁷⁾.
3. Determination of serum IGFBP-3 level using the DSL-10-6600 ACTIVE™ IGFBP-3 ELISA (Diagnostic System Laboratories, Webster, TX) ⁽¹⁸⁾.
4. Determination of serum IGF-1 using a two-site immunoradiometric assay (IRMA) principle. The DSL-5600 IGF-1 (IRMA, Diagnostic System Laboratories, Webster, TX) ⁽¹⁹⁾.
5. Estimation of serum glycoprotein concentrations were determined using a commercial ELISA kit (Myobiosource ELISA; Myobiosource LLC, San Diego) ⁽²⁰⁾.

Statistical analysis:-

Obtained data were presented as mean \pm SD, ranges, numbers and ratios. Results were analyzed using paired t-test, one-way ANOVA with post-hoc Tukey HSD Test and Chi-square test (χ^2 test). Possible correlations were evaluated using Spearman's correlation coefficient and these correlations were verified as sensitivity and specificity for prediction of EPL 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. Data were analyzed using Regression analysis (Stepwise Method) to define the persistently significant predictors for occurrence of EPL among PCOS pregnant women. Statistical analysis was conducted using the IBM SPSS (Version 23, 2015) for Windows statistical package. P value <0.05 was considered statistically significant.

Results:-

During the observation period 58 primigravida PCOS women developed pregnancy loss and so were eligible for evaluation, 13 were excluded and 45 were enrolled in the study as Study group. Another 45 primigravida PCOS women completed the observation period uneventfully and were included as Control group (Fig. 1).

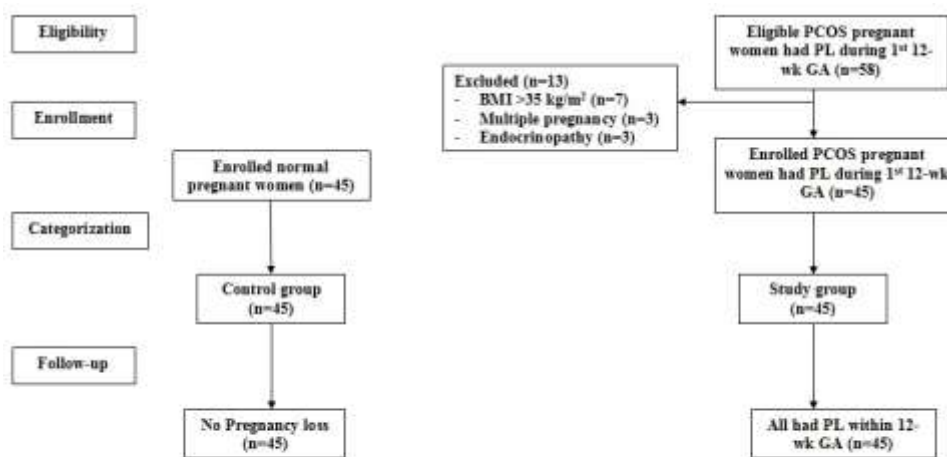


Fig. (1): Flow chart of the study

Women of the study group were found to have significantly higher body weight, fasting blood glucose and HOMA-IR score than control women. Moreover, the frequency of women had IR was significantly higher among women of the study versus the control group. Other evaluated constitutional data showed non-significant difference between women of both groups (Table 1).

Table 1:- Patients' enrollment and IR data

Data	Control group (n=45)	Study group (n=45)	P value
Age (years)	26.2±4.5	25.8±4.8	0.492
Weight (kg)	89±13.5	90.8±13.9	0.021
Height (cm)	165.2±3	166±3.2	0.735
Mean body mass index (kg/m ²)	32.6±5	32.4±5.2	0.562
Fasting blood glucose (mg/dl)	114.1±17.5	118.6±18.8	0.008*
Fasting plasma insulin (mg/ml)	4.9±1.47	5.24±1.57	0.222
HOMA-IR	<2	34 (75.6%)	0.046
	>2	11 (24.4%)	
Mean HOMA-IR	1.49±0.49	1.95±0.55	0.006

Data are presented as mean±SD; *: significant difference

Estimated 4-wk GA serum levels of IGF-1 were significantly higher, while serum IGFBP-3 and Glycodelin A were significantly lower in women of study versus control PCOS women (Table 2).

Table 2:- Laboratory data estimated at time of enrolment

Parameter	Control group (n=45)	Study group (n=45)	P value
Serum IGF-1 (ng/ml)	121.8±47.5	187.6±57.8	0.001
Serum IGFBP-3 (ng/ml)	170.8±34.9	117.4±33.6	0.001
Glycodelin (ng/ml)	636.4±110	484.1±95.4	0.001

Data are presented as mean±SD

Development of EPL in primigravida PCOS women showed positive significant correlation with high FBG, HOMA-IR score and high serum IGF-1 levels, while showed negative significant correlation with serum IGFBP-3 and glycodelin A levels (Table 3).

Table 3:- Correlation between baseline data collected at the 4th wk GA and possibility of EPL in PCOS women

Variable	Rho	P
Age (years)	0.086	>0.05
Weight (kg)	0.171	>0.05
Mean body mass index (kg/m ²)	0.069	>0.05
Fasting blood glucose (mg/dl)	0.255	0.015
Fasting plasma insulin (mg/ml)	0.090	>0.05
HOMA-IR score	0.078	0.035
Serum IGF-1 (ng/ml)	0.578	<0.001
Serum IGFBP-3 (ng/ml)	-0.710	<0.001
Glycodelin (ng/ml)	-0.802	<0.001

Rho: Spearman's correlation coefficient; p<0.05 indicates significant and p>0.05 indicates non-significant correlation; negative mark indicates negative correlation

ROC curve analysis of variable correlated with pregnancy loss defined high serum IGF-1 as significant sensitive predictor, while low serum glycodelin A, IGFBP-1 and FBG as specific predictors with decreasing order of significance (Table 4, Fig. 1).

Table 4:- ROC curve analysis of baseline levels of laboratory parameters estimated at the 4th wk GA as early predictors for pregnancy loss

Variable	AUC (±SE)	p	95% CI
Fasting blood glucose (mg/dl)	0.353 (±0.058)	0.016	0.240-0.466
Serum IGF-1 (ng/ml)	0.166 (±0.042)	<0.001	0.085-0.248
Serum IGFBP-3 (ng/ml)	0.910 (±0.032)	<0.001	0.847-0.973
Glycodelin (ng/ml)	0.962 (±0.016)	<0.001	0.931-0.994

AUC: Area under curve; CI: Confidence interval; p<0.05 indicates significant difference versus the null hypothesis that AUC=0.05

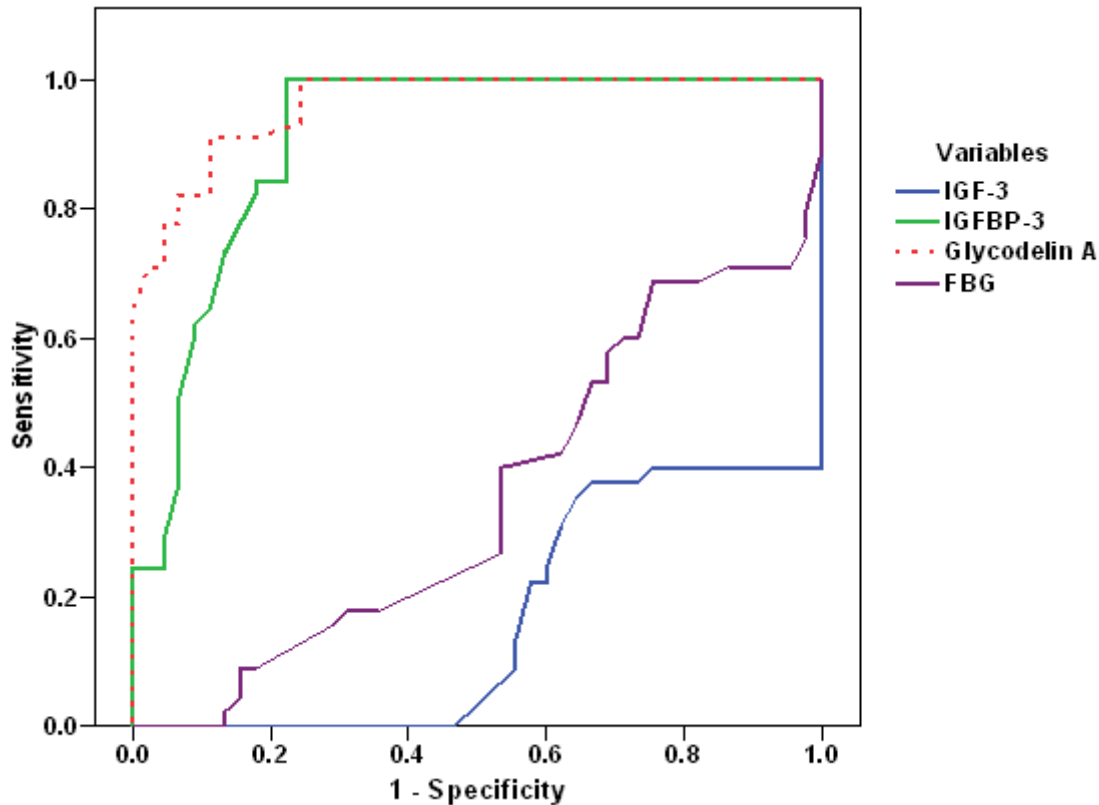


Fig. 2:- ROC curve analysis of laboratory parameters estimated at the 4th wk GA as predictors for PL in pregnant PCOS women

Regression analysis defined low serum glycodelin A levels, decreased serum IGFBP-3 levels and high IGF-1 serum levels as the significant predictors for EPL in primigravida PCOS women in decreasing order of significance (Table 5).

Table 5:- Regression analysis of serum levels of laboratory parameters estimated at the 4th wk GA as predictors for pregnancy loss

Parameter	Model 1		Model 2		Model 3	
	β	p	β	p	β	p
Glycodelin (ng/ml)	0.508	<0.001	0.581	<0.001	<0.784	<0.001
Serum IGFBP-3 (ng/ml)	0.419	<0.001	0.443	<0.001		
Serum IGF-1 (ng/ml)	-0.165	0.006				

β : Standardized Coefficient; p<0.05 indicates significant β value

In trial to define a helpful guide for prediction of PL in PCOS pregnant women, Kaplan-Meier analysis defined serum values of 613.9, 158.1 and 198.8 ng/ml as the cutoff points for glycodelin A (Fig. 2), IGFBP-3 (Fig. 3) and IGF-1 (Fig. 4) serum levels estimated at the 4th week GA to predict a high possibility for PL (Table 7).

Table 7:- Kaplan-Meier analysis for cutoff points of estimated laboratory parameter for prediction of PR among primigravida PCOS women

Parameter	Mean	SE	95% CI
Glycodelin (ng/ml)	613.882	14.871	584.734-643.029
Serum IGFBP-3 (ng/ml)	158.146	4.918	148.506-167.785
Serum IGF-1 (ng/ml)	198.823	6.724	185.644-212.003

SE: Standard error; CI: Confidence interval

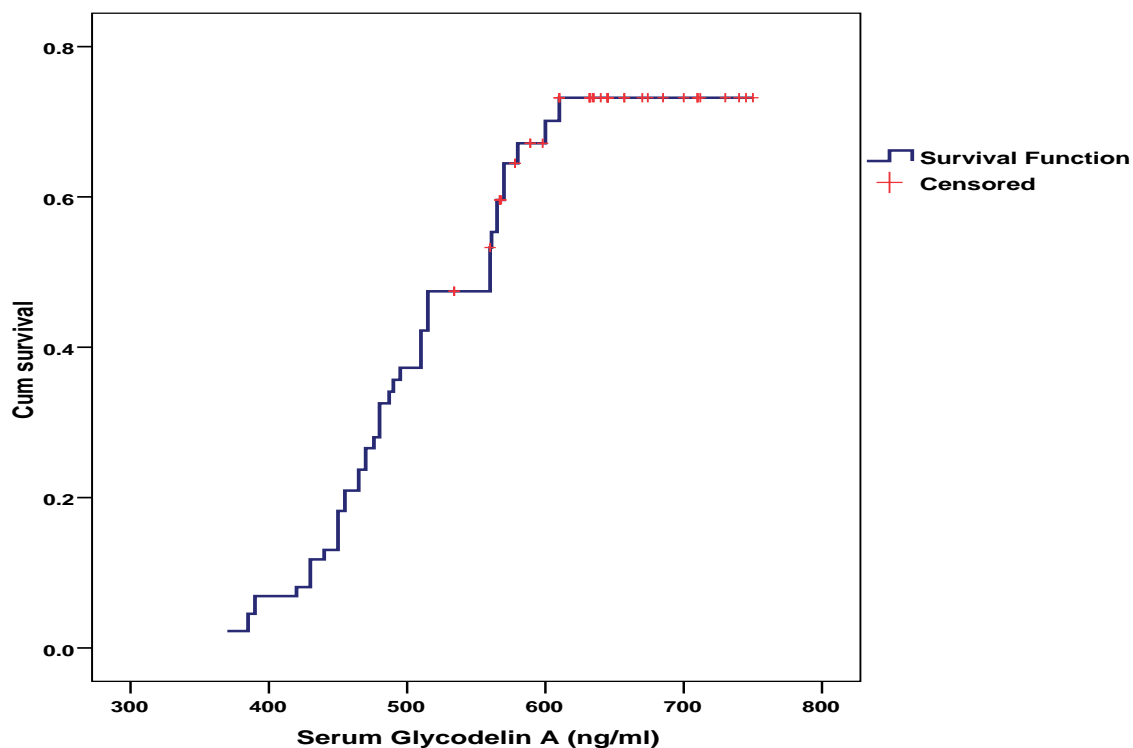


Fig. 3:- Cumulative fetal survival plot according to serum GdA levels estimated at the 4th wk GA showing 612 ng/ml as cutoff point for differentiation between possibility to have PL or continued pregnancy after the 12th wk GA in pregnant PCOS women

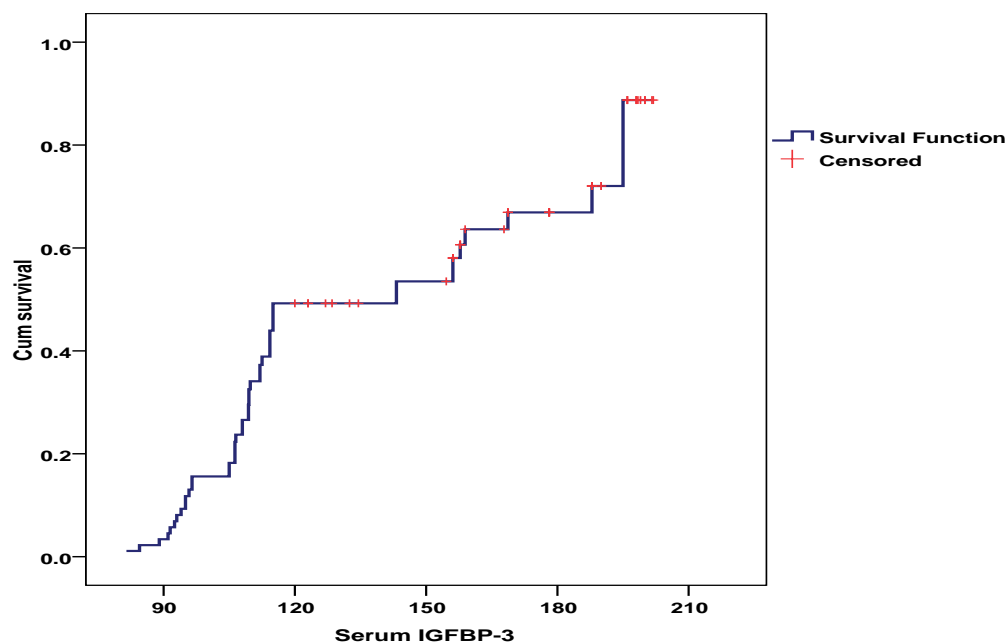


Fig. 4:- Cumulative fetal survival plot according to serum IGFBP-3 levels estimated at the 4th wk GA showing 158 ng/ml as cutoff point for differentiation between possibility to have PL or continued pregnancy after the 12th wk GA in pregnant PCOS women

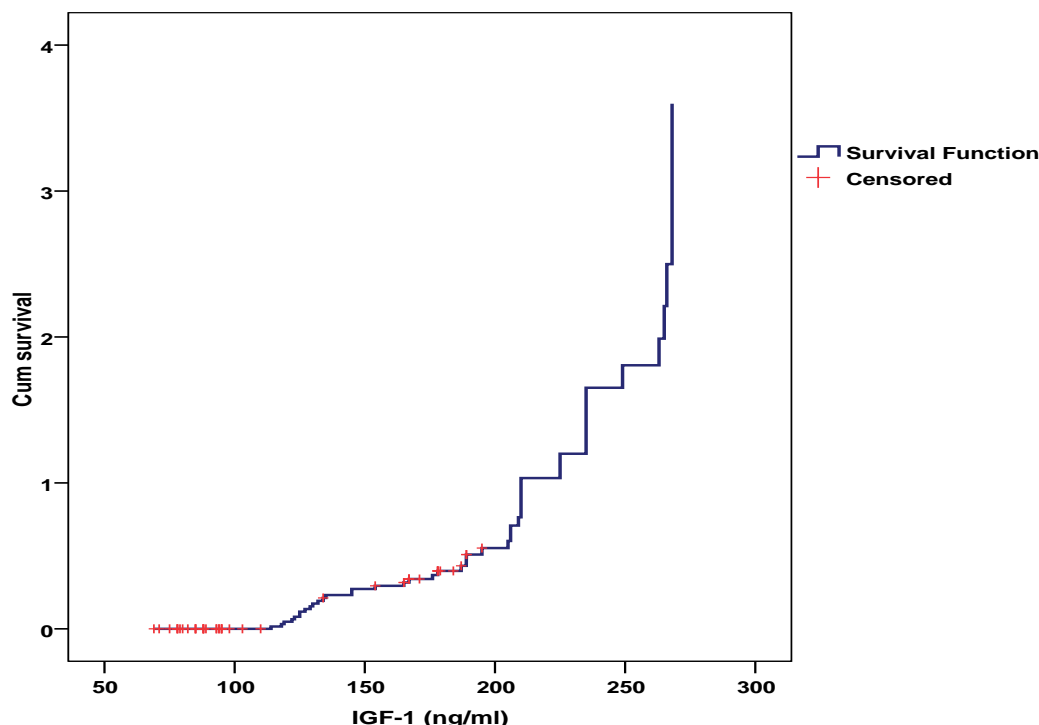


Fig. 5:- Cumulative fetal survival plot according to serum IGF-1 levels estimated at the 4th wk GA showing 198 ng/ml as cutoff point for differentiation between possibility to have PL or continued pregnancy after the 12th wk GA in pregnant PCOS women

Discussion:-

Early pregnancy loss (EPL) is a disastrous state with depressing psychological impacts on affected women especially if recurrent. Infertile PCOS women are more vulnerable to EPL as documented by **Nawaz & Rizvi** ⁽²¹⁾, **Al-Biate** ⁽²²⁾ and **Banu et al.** ⁽²³⁾ who reported EPL rates of 36%, 49.4% and 75%, respectively, among pregnant women with PCOS. **Joham et al.** ⁽²⁴⁾ also documented that PCOS was not independently associated with EPL and multiple coincident factors and events may predispose to or precede the development of EPL, so, the current study aimed to evaluate the predictability of estimation of serum levels of GdA, IGF-1 and IGFBP-3 at the 4th week of gestation for development of EPL in pregnant women with PCOS.

Women of the study group showed significantly higher FBG and HOMA-IR score with significantly higher frequency of women with high HOMA-IR score compared to control women. These findings indicated a relationship between PCOS and both development and persistence of IR despite of control of PCOS-induced hormonal disturbances as indicated by being pregnant. In line with these findings, **Wang et al.** ⁽²⁵⁾ found women with a history of recurrent pregnancy loss (RPL) are at an increased risk for IR during the first trimester of a new pregnancy and **Cho et al.** ⁽²⁶⁾ reported that IR and PCOS are coincident events despite of the higher and more variable score in women with anovulatory than women with ovulatory PCOS. Also, **Celik et al.** ⁽²⁷⁾ documented that compared with the control group; patients with RPL were more likely to have IR. **Maryam et al.** ⁽²⁸⁾ observed significant difference in fasting insulin levels between PCOS and control women with RPL and **Li et al.** ⁽²⁹⁾ reported that IR is associated with the susceptibility to RPL and may contribute its occurrence. Thereafter, **Hong et al.** ⁽³⁰⁾ found woman with RPL who had no pre-conception abnormal glucose metabolism were liable to develop IR during first trimester pregnancy than healthy women, so considered IR as a direct cause for RPL.

Four-wk GA serum IGF-1 levels were significantly higher with concomitant significantly lower serum levels of IGFBP-3 in women of study versus control PCOS women. Moreover, statistical analyses defined high serum IGF-1 as significant sensitive, while low IGFBP-1 and high FBG as specific significant predictors for EPL in pregnant PCOS women. In line with these findings, **Kelly et al.** ⁽³¹⁾ reported significantly lower serum IGFBP-1 levels in

PCOS women than controls and **Luo et al.**⁽³²⁾ found that miscarried PCOS patients showed significantly increased free IGF index with decreased IGFBP-3 than in pregnant women had PCOS and a successful pregnancy.

These findings indicate less utilization of IGF-1 with subsequent decrease of its growth promoting effects on uterine tissues leading to impaired placentation, decidual invasion and defective embryo implantation that may facilitate induction of pregnancy-related complications up to EPL. In support of this assumption, **Brazert & Pawelczyk**⁽³³⁾ using PCR detected the transcripts of the three IGF-1 isoforms in ovarian tissue of healthy controls and PCOS patients, but expression of all isoforms was lower in control women compared to PCOS women and concluded that PCOS is associated with increased ovarian expression of IGF isoforms that may underlie pathogenesis of PCOS. Thereafter, **Luo et al.**⁽³²⁾ detected significantly increased expression of IGF-1 and decreased IGFBP-1 in the deciduas of miscarried PCOS patients compared with non-PCOS and suggested that early miscarriage is associated with increased IGF-1 and decreased IGFBP-1 in PCOS patients. Recently, in 2018, **Zheng et al.**⁽³⁴⁾ supposed that excessive expression of the transcription factor PAX6 in insulin-challenged endometrial epithelial cells may contribute to the uncontrollable endometrial epithelial proliferation that may predispose to EPL in women with PCOS.

Estimated 4-wk GA serum levels GdA were significantly lower in women of study versus control PCOS women, are negatively and significantly correlated with possibility of development of EPL and statistical analyses defined low serum GdA as the highly significant specific predictor for EPL in primigravida PCOS women. These findings go in hand with **Giudice**⁽³⁵⁾ who detected decreased levels of biomarkers of endometrial receptivity to embryonic implantation, as GdA in PCOS women and with **Toth et al.**⁽³⁶⁾ who indicated that significantly reduced GdA expression is associated with miscarriage. Also, **Bastu et al.**⁽³⁷⁾ detected significantly lower blood and tissue measurements of GdA in women with recurrent implantation failure than in fertile women with a highly significant correlation between blood and tissue levels of GdA.

In support of the high predictability of estimated serum GdA for possibility of having EPL, multiple studies assured the determinant role of GdA for adequate endometrial receptivity, proper placentation and decreased chance of PL where **Li et al.**⁽³⁸⁾ reported that GdA levels in uterine flushing of women with good uterine receptivity was significantly higher and rate of clinical pregnancy and term delivery were significantly high with significantly lower spontaneous abortion rate than in women with poor receptivity. **Tapia-Pizarro et al.**⁽³⁹⁾ found immunostaining for GdA was significantly decreased in endometrial samples from women experienced repeatedly implantation failure. Also, **Uysal et al.**⁽⁴⁰⁾ reported that when GdA expression was high in PCOS group, the pregnancy rate was 60% and all pregnancies ended in live births, while in weak expression group, EPL rate was 75% and concluded that endometrial GdA expression is an important predictor of pregnancy outcomes in both PCOS and fertile groups. **Lee et al.**⁽⁴¹⁾ attributed such role of GdA to the findings that GdA interacts by its unique carbohydrate side chains with the cell surface of various cell types in the human fetomaternal interface and modulates their functions and differentiation to permit successful pregnancy, and documented that abnormal GdA levels in the endometrium, uterine flushings, and/or maternal serum correlate with unexplained infertility, early and recurrent pregnancy loss.

The relationship of increased frequency of EPL with low serum IGFBP-1 and GdA levels on one side and high serum IGF-1 level on the other side spot light on a relation between disturbed endometrial receptivity and growth controlling factors as a pathogenic basis for EPL and indicated the value of estimation of an array of markers to predict EPL especially in this vulnerable group of infertile women. In line with these assumptions, **Douglas et al.**⁽⁴²⁾ reported that the GdA/IGFBP-1 ratio on the day of blastocyst transfer was higher in recipients who achieved pregnancy. **Senapati et al.**⁽⁴³⁾ documented that multiple marker panels especially including GdA aiming to maximize sensitivity and specificity results in high accuracy for distinguishing pregnancy location and viability in women at risk for early pregnancy complications.

Conclusion:-

Low serum GdA and IGFBP-3 levels and high IGF-1 levels estimated at the 4th wk GA may underlie the development of EPL in pregnant PCOS women. Estimation of three markers as multiple marker panels could predict EPL with high sensitivity and specificity. However, the supposed cutoff points need to be confirmed through wide scale comparative study.

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