Maternal Serum Placental Leucine Aminopeptidase as a Predictor of Pregnancy Outcomes

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ABSTRACT:

BACKGROUND:

Placental leucine aminopeptidase is a glycoprotein, known as oxytocinase. It helps to maintain normal pregnancy to term, also it is involved in fetal development, maintenance of homeostasis during pregnancy, blood pressure regulation, memory retention, antigen presentation, and cancer development.

OBJECTIVE:

To evaluate the value of maternal serum placental leucine aminopeptidase levels as a predictor of pregnancy outcomes.

PATIENTS AND METHODS:

Hundred pregnant women were included in the study, they were divided into four groups, 25 pregnant women with hypertensive disorder of pregnancy, 25 pregnant women with gestational diabetes and 25 pregnant women with intrauterine fetal death at a gestational age between 28 - 41 weeks, another 25 healthy pregnant women as a control group with gestational age range 37 - 40 weeks. Serum levels of placental leucine aminopeptidase were measured using Enzyme-linked immunosorbent assay (ELISA) method for all study groups at the time of presentation. It is a case - control study that was conducted at AL-Yarmook Teaching Hospital, department of obstetrics and gynecology.

RESULTS:

Serum placental leucine aminopeptidase concentration significantly lower in pregnant women presented with fetal death group was(mean ± SD)61.6 ± 15.8IU/ml compared to pregnant women with hypertensive disorders in pregnancy, gestational diabetes mellitus and healthy pregnant women were (83.2 ± 12.51IU/mL, 85.0 ± 13.11IU/mL and 108.9 ± 19.1IU/mL), respectively. Receiver operating characteristic(ROC) curve analysis showed that the result of serum placental leucine aminopeptidase level for predicting perinatal mortality among pregnant women was (optimal cut point ≤78.98, sensitivity100%, specificity 73.3and accuracy 80.0%).

CONCLUSION:

Maternal serum placental leucine aminopeptidase was low among patients with hypertensive disorders in pregnancy, gestational diabetes mellitus and much lower among patients presented with fetal death compared to healthy pregnant women. Low serum placental leucine aminopeptidase level had high sensitivity and specificity for predicting perinatal mortality.

KEYWORDS: placental leucine aminopeptidase, hypertensive disorders of pregnancy, gestational diabetes mellitus and fetal death.

INTRODUCTION:

The pregnancy outcome is the final result of a fertilization event[1].Adverse pregnancy outcomes are influenced by a number of biological, social and environmental factors[2].

Placental Leucine Aminopeptidase (P-LAP) also known as oxytocinase is a glycoprotein secreted by syncytiotrophoblast[3]. P-LAP was first synthesized in the placenta as a membrane protein and then secreted into the maternal serum.

P-LAP activity in serum remains low in the first trimester[4], (PLAP) is helping to maintain normal pregnancy to term[5]. LAP present in all human sera and during pregnancy originates from the placenta so-called P_LAP[6].

Human gestation is characterized by altered responses to exogenously infused peptide hormones include oxytocin, vasopressin, and angiotensin[4].

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The pregnant uterus is sensitive to exogenously infused oxytocin at term compared with the second and third trimester[4].Therefore, placental leucine aminopeptidase (P-LAP) plays a major role in the clearance of oxytocin, which is a key hormone in regulating labor pain[7].

There is evidence that oxytocinase and other placental aminopeptidases affect the altered response of pregnant women to exogenously infused peptide hormones via their degradation whose increase might have a significant effect on uterine and utero-placental blood flow via regulating oxytocin levels[4].

P-LAPis referred to as insulin-regulated membrane aminopeptidase associated with the glucose transporter 4-containing vesicle[8]. It is translocated to plasma membrane by insulin stimulation in adipocytes and skeletal muscle cells, suggesting it has roles in the pathogenesis of diabetes[9].

The change of balance between fetal oxytocin (OT)/vasopressin (AVP) and P-LAP due to fetal growth, and in response to various stimuli such as hypoxia in the fetoplacental unit, likely triggers the onset of labor including preterm labor [10,11].

AIM OF THE STUDY:

To evaluate the value of maternal serum placental leucine aminopeptidase levels as a predictor of pregnancy outcomes.

PATIENTS AND METHODS:

This is a case-control study was conducted in the department of Obstetrics and Gynecology of AL-Yarmouk Teaching Hospital from March through November 2017. The study protocol was approved by the Scientific Council of Obstetrics and Gynecology specialization /Iraqi Board for Medical Specializations. The study included 100 pregnant women who attended the department of Obstetrics and gynecology for delivery.

They were informed about the nature of the study and verbal consent was obtained from them. The women included in the study were divided into four groups: Group A:25 pregnant women with hypertensive disorders of pregnancy(HDP) .Group B: 25 pregnant women presented with Gestational diabetes mellitus.

Group C: 25 pregnant women with Intrauterine fetal death or demise. Group D (control group): 25 healthy pregnant women with full-term pregnancy without any complications throughout the pregnancy.

The inclusion criteria: singleton viable and non-viable pregnancy, gestational age ranging between 28 - 41 weeks confirmed by obstetrical criteria depending on accurate last menstrual period and / or by early ultrasonography and any maternal age and parity status. The exclusion criteria: multiple pregnancies, other maternal or chronic diseases affecting the pregnancy other than hypertension and GDM.

Detailed history was obtained, general and obstetrical examination was done and routine and specific investigation were done according to the case included in the study. after delivery, recorded information included type of delivery(vaginal or cesarean) and neonatal outcome obtained from pediatrician include; newborn sex,1-minute and 5-minute Apgar scores and birth weight.

Sample collection and assay:

Venous blood sample of 5ml were collected in a plain tube from each patient, the sample allowed to clot for 10-20 minutes at room temperature, centrifuge at (2000-3000RPM) for 20 minutes, collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again. Serum separated and stored at -60C or below until the analysis was performed. An enzyme-linked immunosorbent assay (ELISA) kit was used for this purpose.

Statistical Analysis

The data analyzed were done by using Statistical Package for Social Sciences (SPSS),version 17, data were expressed as mean ± SD (standard deviation of the mean or number), frequency & percentage. Continuous normally distributed variables were compared by analysis of variance test (ANOVA) for more than one group.

Student t test used for difference between two means, while different percentages were tested using Pearson Chi-square X^2 tests. Receiver Operator Curve (ROC) was used to assess the sensitivity and specificity of serum P-LAP levels to predict pregnancy outcomes. P - Value less than 0.05 was considered to be statistically significant.

RESULTS:

The current study showed no significant difference among the different groups in their age and parity, while gestational age shows in group C had a significant lower gestational age compared to group A, B and D.

Table 1: Demographic data of maternal characteristics

Variables	Group A (n=25)	Group B (n=25)	Group C (n=25)	Group D (n=25)	P value
Age (years); mean ± SD	28.88 ± 5.80	28.96 ± 5.14	29.40 ± 5.92	29.88 ± 5.22	0.914
Parity; mean ± SD	2.4 ± 2.4	1.9 ± 1.6	2.1 ± 1.7	2.3 ± 1.8	0.791
Gestational age (weeks); mean ± SD	36.96 ± 3.27	36.79 ± 2.73	34.47 ± 3.45	38.47 ± 1.13	<0.001
$28 – 36^{+6}$	9 (36.0%)	8 (32.0%)	17 (68.0%)	0 (0.0%)	
37 – 40	10 (40.0%)	15 (60.0%)	6 (24.0%)	25 (100.0%)	
$>40^{+1}$	6 (24.0%)	2 (8.0%)	2 (8.0%)	0 (0.0%)	

[a] One way ANOVA , SD= standard deviation

As shown in table 2, P-LAP level in group C was significantly lower compared to group A, B and D groups ($p<0.001$).While no significant difference among A and B ($p<0.001$).

Table 2: Maternal P-LAP (IU/ml) in the study groups.

Variables	Group A (n=25)	Group B (n=25)	Group C (n=25)	Group D (n=25)	P value
P-LAP Mean ± SD	83.2 ± 12.5	85.0 ± 13.1	61.6 ± 15.8	108.9 ± 19.1	<0.001

One way ANOVA, SD= standard deviation

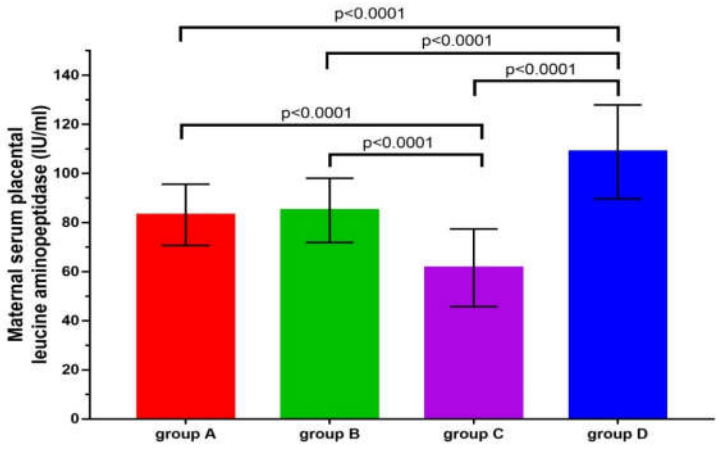

Figure 1: maternal serum placental leucine aminopeptidase concentration in the study groups.

Note that each pair without p value mean it is ≥0.05.

The predictive value of serum P-LAP concentrations in perinatal mortality (AUC between 0.8 – 0.89) with optimal cut point ≤78.98, The sensitivity and specificity of the study were 100% and 73.3%respectively, so that (sensitivity> specificity)for predicting perinatal mortality, as shown in table 3

Table 3: Validity of P-LAP for predicting of perinatal mortality

AUC	P value	Cut point	Sensitivity	Specificity	Accuracy	PPV	NPV
0.906	<0.001	≤78.98	100%	73.3%	80.0%	55.6%	100%

AUC: area under the curve, PPV: positive predictive value, NPV: negative predictive value

ROC uses the change in sensitivity (SN) and specificity (SP) for each point of the investigated marker (p-LAP) and create curve, and we measure the area created from this curve (and found its percent from 100%), the optima cut – off calculated using:

Youden index

The Youden index **J** (Youden, 1950) is defined as:

J = max { sensitivityc + specificityc - 1 }

where c ranges over all possible criterion values. Graphically, J is the maximum vertical distance between the ROC curve and the diagonal line.

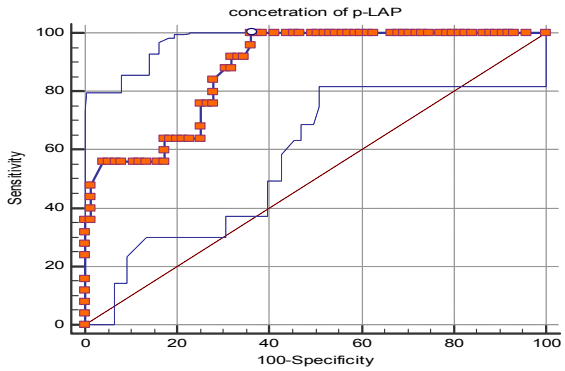

Figure 2:Receiver operating characteristic(ROC) curve show the value of serum P-LAP concentrations in predicting perinatal mortality.

Women with gestational age (28 – 36^{+6}) weeks: Group C had significant lower P-LAP compared to group A and B (p= 0.041). In women with gestational age (37 – 40) weeks group D had significant higher P- LAP concentration compared to group A, B and C, while no significant difference between group A and B (p<0.001). In Women with gestational age >40^{+1}weeks, group C had significant lower P-LAP compared to group A and B (p= 0.019),which means that the P-LAP level in group C was lower than the other study groups at all gestational age as illustrated in table 4.

Table 4: Maternal P-LAP in the study groups stratified according to gestational age at delivery.

Variables	Group A (n=25)	Group B (n=25)	Group C (n=25)	Group D (n=25)	P value
Number	***9***	***8***	***17***	***0***	-
28–36^{+6} mean ± SD	76.62±8.03	70.35 ± 5.01	61.80 ±18.12	-	0.041
Number	***10***	***15***	***6***	***25***	-
37–40 mean ± SD	92.05±13.34	94.13 ± 7.19	62.64±11.75	108.86±19.14	<0.001
Number	***6***	***2***	***2***	***0***	
>40^{+1} mean ± SD	78.19 ± 7.65	75.07 ± 6.58	56.65 ± 1.41	-	0.019

One way ANOVA, SD= standard deviation

The regression analysis between p-LAP is illustrated in the table 5

Table 5: Correlation between p-LAP and various variables

Variables	Group A		Group B		Group C		Group D	
	β	P value	β	P value	B	P value	β	P value
Gestational age	0.293	0.156	**0.561**	**0.004**	0.335	0.102	**0.438**	**0.028**
APGAR1	**0.625**	**0.001**	**0.398**	**0.049**	-	-	0.348	0.089
APGAR5	**0.598**	**0.002**	**0.571**	**0.003**	-	-	0.292	0.157

β: correlation coefficient

We notice for group B and D p-LAP is affected by gestational age (direct relationship) while for groups A and C, no such relationship is present.

DISCUSSION:

Placental leucine aminopeptidase (P-LAP) is the only membrane aminopeptidase known to functionally degrade oxytocin as oxytocinase during pregnancy[12].It is usually found in the soluble form in the mother and in the membrane – bound in the placenta [13]. The fetus actively produces oxytocin from the early weeks of gestation and it continues to produce in a matter that do not affect maternal endometrial production of P-LAP in its soluble form[14].

The current study shows that the measurement of serum P-LAP was significantly lower in women presented with (HDP, GDM and fetal death) compared to healthy pregnant women at term pregnancy who had similar gestational age (P<0.001).

In a study done by **H. Kozaki et al**[15]**,** they studied 61 pregnant women without complications were recruited to determine normal reference values for P-LAP activity in pregnancy, they were divided as preterm and term delivery groups. They found no significant differences with respect to maternal age, parity and rate of cesarean delivery or gender group, this agreed with the current study.

Regarding P-LAP level in maternal serum increases with advancing pregnancy, When P-LAP activity was at or below the 10th percentile, they found a greater ratio of preterm delivery (P=0.0085) and approximately 2.3-fold increase risk. The sensitivity, specificity, positive predictive value and negative predictive value were 29, 97, 89 and 62%, respectively. This result disagrees with the current study in which P-LAP level had high sensitivity and specificity with positive predictive values and negative predictive values were (100%, 73.3%, 55.6% and 100%)respectively, this may be due to pregnant women with HDP,GDM and fetal death were included in thestudy.

Regarding the relationship between the serum P-LAP level and the gestational age. **Anuradha et al**[16], studied the serum (P-LAP) level in 60 pregnant women (30 healthy pregnant women as a control group and 30 cases with hypertensive disorder of pregnancy HDP) with gestational age range between (28-40)Weeks.

They found the mean value of P-LAP levels in the control group with a gestational age range between 28-$36^{+6}$and 37-40 were (64.016 and 91.12U/L) respectively, while the mean value of P-LAP levels in women with HDP for the same gestational age were (29.46 and 39.76U/L), respectively.

They concluded that serum P-LAP will continue to increase normally as pregnancy progress while in the HDP this gradual increase is interrupted and it will remain lower than that of the control. This is in agreement with the current study which showed that serum P-LAP level in the control group was (108.86 ± 19.14 IU/ml) at gestational age 37 – 40 weeks, while serum P-LAP level in HDP women was higher at gestational age 37 – 40 weeks (92.05 ± 13.34 IU/ml) compared to 28 – $36^{+6}$and $>40^{+1}$ week (76.62 ± 8.03 IU/ml and 78.19 ± 7.65 IU/ml),respectively.

S. A. Mohamed et al[17], studied serum P-LAP level in 26 healthy pregnant women with a gestational age range between 26 to 38 weeks who were delivered vaginally of a single healthy baby of 2.5 kg or more, while in the current study P-LAP level was measured in healthy pregnant women with a gestational age range between 37 to 40 weeks who were delivered vaginally or by caesarian section.

They found that P-LAP activity in maternal sera increases progressively with advancing gestation with a positive correlation between P-LAP levels and gestational age (r = 0.61, p <0.001).Which agrees with the current study (p <0.001).

In another study done by **Tian et al** [18],they measured serum P-LAP for 117 pregnant women (38 pregnant women with HDP, 35 with GDM and14 experienced intrauterine fetal death with a gestational age range between 28-41 weeks and 30healthy pregnant women with a gestational age range between37-40 weeks as a control group).They found that the gestational age of fetal death group was shorter than among women in each of the HDP, GDM and control group(P=0.045, P=0.048, and P=0.036) respectively, which is in agreement with the present study (P<0.001).

Regarding the mode of delivery they found no significant differences among the four groups which disagree with the present study, cesarean section was significantly lower than vaginal delivery in the fetal death group compared with other study groups (P=0.002), also a higher rate of cesarean section was found in HDP and GDM, this may due to the condition of some patients at the time of the admission to the delivery room or because of having previous one cesarean section or more which increased rate of cesarean section in the current study.

They found the newborn Apgar scores at 1 and 5 minutes did not differ significantly among the HDP, GDM and control group but were regarded as 0 in the fetal death group, which agrees with present study[18].

The maternal serum P-LAP levels in the HDP, GDM and fetal death groups in the above study were significantly lower than those in the control group (P=0.031, P=0.042, and P<0.001) respectively, while serum P-LAP levels were significantly higher in the HDP and GDM groups than in the fetal death group (both P<0.001), also they found that serum P-LAP levels among women in the fetal death group remained low at all pregnancy lengths. Which agrees with current study, where serum P-LAP level was higher in the control group compared to the other study groups(P<0.001).

Also, they found that the optimal cutoff value of serum P-LAP level for predicting perinatal mortality was 47.07 U/L, the sensitivity and specificity were (100% and 78.9%), respectively[60].This is in agreement with current study in which optimal cut point ≤78.98, the sensitivity and specificity were (100% and 73.3%),respectively.

Several studies done on animals to evaluate the role of P-LAP in several conditions. Experimental study on rats done by **Mizutani et al**, they investigated the role of P-LAP in the prediction of preeclampsia and preterm labor. Because P-LAP has a large molecule (molecular weight of 160–165 kDa) that do not cross the placental barrier, they found that serum P-LAP level can be used as a promising agent to treat preeclampsia and preterm labor by degrading bioactive hormones derived from fetoplacental circulation and may be useful in avoiding the onset of midnight labor, which is troublesome for all obstetricians[10].

A recent study done by **KINOSHITA et al.**, on female Bornean orangutans. They examined the differences in urinary P-LAP concentrations between live births and a stillbirth, they found during late pregnancy the mean daily P-LAP activity progressively increases. On the other hand, it was reported that P-LAP gradually decreased when intrauterine fetal death occurred[19].

CONCLUSIONS:

Maternal serum placental leucine aminopeptidase was low among patients with hypertensive disorders in pregnancy, gestational diabetes mellitus and much lower among patients presented with fetal death compared to healthy pregnant women. Low serum placental leucine aminopeptidase level had high sensitivity and specificity for predicting perinatal mortality.

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