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Research Article

IMPACT OF SERUM PLACENTAL ALKALINE PHOSPHATASE (PALP) LEVEL ON ABORTION

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ABSTRACT

Objective of this study is to evaluate the impact of placental alkaline phosphatase on abortion in the second trimester through determining the changes in serum levels of routine liver function tests. Evaluation was done by using sera of 41 abortion women at second trimester (patients group), as well as 39 healthy normal pregnant women at second trimester involved as control. Data analysis shows no significance between age, gestational age, liver transaminases enzymes and total protein of patients and control women. A highly significant decrease in placental alkaline phosphatase isoenzyme level was found in patients compared with the control ($p < 0.001$). Both total alkaline phosphatase and placental alkaline phosphatase of abortion women were highly specific and sensitive. In conclusion, total alkaline phosphatase and Placental alkaline phosphatase levels in each case of abortion women at 2nd trimester in addition to their highly specificity and sensitivity may help the gynecologist in predicting abortion especially when these enzymes were estimated at the middle of 2ndtrimester (20 weeks of gestation).

Keywords: placental alkaline phosphatase, second trimester abortion, liver function tests.

INTRODUCTION

Alkaline phosphatase (ALP) is present in many tissues of the body with particularly high levels in the liver, bone, placenta, intestine and kidney.[1]

It is elevated in serum during pregnancy, especiallyin the second and third trimesters. Normally, serum totalalkaline phosphatase (TALP) begins to rise at the fourth month of gestation.[2] This elevation is a reflection ofplacental ALP entering the maternal blood, since humantrophoblastic cells are rich in ALP, measurement ofserum placental ALP origin is, therefore, of particularinterest in the investigation of placental insufficiency.[3] Also, extremely high serum TALP concentrations shouldarouse suspicion of bone, hepatic, endocrine, renal, ormalignant diseases.[1]

A research done by Aleemin 1972 suggested that the increase in alkaline phosphatase activity may accompany threatened abortion.[4] The term abortion means loss of the fetus, for any reason, before it is able to survive outside the womb.[5] It covers accidental or spontaneous ending, or miscarriage, of pregnancy as well as deliberate termination.[6]

While very low activities of total alkaline phosphatase in first trimester indicated affected fetus (Muller et al., 1991).[7] In other hand, Rosenau et al. (1994) reported greater elevation of total alkaline phosphatase in cases of vomiting during first trimester.[8]

Duringpregnancy, the serum sex steroid hormones such as estrogen and progesterone increase progressively and reach a maximum during the third trimester,they have effect on metabolic, synthetic, and excretory hepatic functions.[9] The increase in plasma volume that occurs during pregnancy leads to haemodilution and decreases the serum protein concentrations. Since serum alkaline phosphatase levels increase in late pregnancy because of both a production of the placental isoenzyme and an increase in the bone isoenzymes; [10] it is therefore not surprising that changes in liver function tests (LFTs) occur during pregnancy. The identification of these physiological changesis important for the diagnosis of liver diseases during pregnancy.[11]

So, this study was aimed to evaluate the impact of placental alkaline phosphatase on abortion in the second trimester through

determining the changes in serum levels of routine liver function tests, *i.e*., Total and Direct bilirubin, Alanine transaminase (ALT, E.C.2.6.1.2)(GPT), Aspartate transaminase (AST, E.C.2.6.1.1)(GOT), total Alkaline phosphatase (ALP, E.C.3.1.3.1), , Gamma glutamyl transpeptidase (GGT, E.C.2.3.2.2), serum total proteins, Albumin, Globulins and Albumin/Globulin ratio (A/G ratio)in addition to serum Placental Alkaline phosphatase(ALP, E.C.3.1.3.1; isoenzyme) during abortion in second trimestercompared with a control group of the same duration of pregnancy in second trimester pregnant women.

MATERIALS AND METHODS

A case-control study was conducted at different hospitals in Baghdad (Iraq) from April 2102 to March 2015. Forty one abortion women at second trimester was involved in this study as patients group, while thirty nine healthy normal pregnant women at second trimester was considered as control. Both patient and control women age were comparable.

Patients with hypertension, past history of jaundice or pruritus, chronic alcoholism, seropositivity for hepatitis B or VDRL, smoking, evidence of active infective, fever, chronic inflammatory diseases (including rheumatoid arthritis, joint pain, osteoarthritis, abdominal complain, inflammatory bowel disease); currently taking any medication and/or have history of ingestion of hepatotoxic drugs (antituberculosis drugs or anticonvulsants), or having a positive test for cytomegalovirus (CMV) or toxoplasmosis were excluded from this study.

Before blood sampling, all patients and control women were requested to involve in this study. About 10 milliliters of venous blood was taken from patients during their admission to hospitals at the time of their abortion. Also, the same amount of blood was taken from control women at second trimester of pregnancy.

Blood was transferred into a plain tube, allowed to clot at room temperature, and then centrifuged for 1 min at 3000 rpm to collect serum, then it was stored at 2-8° C temperature till analysis was done.

Liver function tests; serum total and direct bilirubin was estimated using CliniChem colorimetric method. Serum alanine transaminase (ALT; GPT) and aspartate transaminases (AST; GOT) concentration with colorimetric method (Biomaghreb). Serum total alkaline phosphatase (TALP) level was measured using Human ALP (Alkaline Phosphatase) ELISA Kit; Elabscience, cat. No. E-EL-H0146). Serum gamma glutamyl transpeptidase (GGT) level was measured by colorimetric method (Human). Serum total protein concentration was estimated by biuret end point method. Serum albumin concentration was measured using colorimetric method (AGAPPE). Serum globulin concentration was calculated by subtracting albumin concentration from total protein concentration. Albumin/globulin ratio (A/Gratio) was also calculated.

Serum Placental Alkaline Phosphatase was measured using Human PLAP/ALPP(Placental Alkaline Phosphatase) ELISA Kit; Elabscience, cat. No. E-EL-H 1976.

Normal reference ranges values for biomarkers at 37 ºC used in this study were as follow; serum total bilirubin, < 1mg/dl; serum direct bilirubin, < 0.3;serum GPT, < 45 U/ml; serum GOT, < 40 U/ml;serum Total ALP (TALP), 3.125-200 ng/ml; serum GGT,9-39 U/l;serum total protein, 3.7-5.3g/dl; serum albumin, 2.3-3.6 gm/dl; serum globulin, 1.0-2.3 g/dl; serum albumin/globulin ratio; 0.8-2;serum placental alkaline phosphatase (PALP); 0.156-10 ng/ml.

Ethical approval and patient permission were obtained from the local ethics committee in Al-Nahrain College of Medicine to conduct this study.

STATISTICAL ANALYSIS

Data were statistically analyzed by SPSS version 17 and Excel 2013. All data were presented as a mean±SE. Statistical differences between value of patients and control groups were determined by student t-test. Correlation between the variables was performed by spearman correlation coefficient. P value < 0.05 was considered as significant.The sensitivity and specificity were calculated for the used kits in classifying the true positive versus false positive cases in this study.

RESULTS

No significance was found regarding age and GA of patients and control women; the age and GA were comparable, and patient's serum total and direct bilirubin concentrations were significantly increased compared with control. (Table-1)

Serum GPT and GOT levels of normal pregnant women were significantly increased compared with abortion, while γ-GT level was highly increased in abortion compared with normal control pregnancy. Both GPT/GOT ratio and total protein were not significant. Serum albumin concentration in abortion women was increased with highly significance compared with normal pregnant women.

Inversely, globulin concentration was significantly decreased in abortion sera compared with normal pregnant women at same second trimester. From these observed values in table-1, serum Alb/Glob ratio was highly significant increase in abortion women at second trimester compared with the normal . Serum TALP and PALP were significantly higher in normal pregnancy compared with abortion although the abortion TALP and PALP levels were within the normal reference range. But the PALP of normal pregnant women was slightly increased more than the upper limit of its reference range. Thus, ratio between PALP/TALP was significantly decreased in abortion compared with normal pregnancy at second trimester .

Additionally, women with total serum ALP less than 53 (95% CI, 50-76) and serum placental ALP less than 2.5 (95% CI, 1.0-2.9) were more likely to became abortion than others.

For women at second trimester abortion, the Pearson correlation and multiple linear regression analysis between anthropometric and biochemical parameters were found significantly differed. A positive significant correlations was found between (age+TSBn; GPT+GPT/GOT ratio; TP+[TSBn, Glob, Alb/Glob ratio]; as well as between PALP [DSBn, PALP/TALP ratio]). While, a negative significant correlations was found between (age+GA; GOT+GPT/GOT ratio and PALP+GA). Multiple linear regression analysiswas used to confirm the independent correlations between these variables.(Table-2)

In the other hand, in table-3; for normal pregnant women at second trimester, the Pearson correlation and multiple linear regression analysis between biochemical parameters were also found significantly differed. A positive significant correlations were found between (GPT+GPT/GOT ratio, GPT+GOT; TP+Glob). While, a negative significant correlations were found between (GOT+GPT/GOT ratio, TALP+[GA, DSBn, PALP/TALP ratio] and Glob+[Alb/Glob ratio, PALP, PALP/TALP ratio]. Multiple linear regression analysis was used to confirm the independent correlations between these variables.(Table-3)

To evaluate the validity of PALP/TALP ratio compared to GPT/GOT ratio, and Alb/Glob ratio, in addition to other LFTs, the probability ratio of a positive test was calculated to confirm the positive significant correlation found as mentioned in Table-4. The sensitivity of PALP/TALP ratio was higher than GPT/GOT ratio and Alb/Glob ratio (69.58, 66.16 and 64.27 respectively). Also, the specificity of PALP/TALP ratio was higher than the rest ratios. In other hand, GPT/GOT ratio was slightly higher than Alb/Glob ratio but still less than PALP/TALP ratio.(Table-4).

DISCUSSION

The present study is the first of kind studied liver function tests in abortion women at second trimester compared with normal pregnancy at same trimester. According to study results, total serum and direct bilirubin concentrations were slightly significantly higher in abortion at second trimester compared with normal pregnancy at same trimester ($p<0.039$ and 0.023 respectively). GohelMGmet al. 2013 reported that concentrations of both total and direct bilirubin were significantly lower in second trimester than first trimester and non-pregnant women.[12] A decrease in serum total bilirubin concentration has already been observed during pregnancy in various studies.[13] Haemodilution could at least partly be responsible for the decrease in bilirubin concentration because albumin is the protein that transports bilirubin.[12]

Concerning serum total protein and its main fractions, serum total protein shows no significant changes in their concentrations except serum albumin and globulin. The highly significance increase in serum albumin concentration was found in abortion compared with normal pregnancy, while the serum globulin was found significantly higher in normal pregnancy at second trimester compared to abortion at same trimester. Gohel MG et al. 2013 mentioned that late in pregnancy, albumin concentration was reduced much compared to early pregnancy,[13] also they reported that the reduction of albumin concentration, combined with a normal slight increase in serum globulin, results in decrease in albumin to globulin ratio similar to that seen in certain hepatic disease.[14] In present study, serum albumin/globulin ratio was significantly reduced in second trimester pregnant women compared to abortion women at same trimester, this might due to the increase of serum albumin in abortion more than in normal pregnancy. This finding is unlike what Cunningham FG in 2005 how explained the decrease in albumin to globulin ratio and considered it as a phenomenon of haemodilution in the interpretation of all serum concentration values of serum total protein during pregnancy.[14]

Regarding liver enzymes, GPT, GOT and γ-GT respectively, it is obvious that both GPT and GOT were both higher in second trimester of normal pregnancy than in abortion at

second trimester and so, their ratio was higher in normal pregnancy compared with abortion. Although all studies concern about levels of liver function tests during normal pregnancy or in pregnancy diseases, *i.e.*, pre-eclampsia, but still abortion no obvious studies was found. So, the lowering in GPT and GOT levels cannot be explained. However, in 1971 and 1989 respectively was mention that serum GPT and GOT levels were higher in late pregnancy than in early pregnancy. Also, it was mentioned that increase in GOT and GPT was found during labour, which might be caused by contraction of uterine muscle,[15,16] and this condition is not comparable with abortion.

In other hand, in the present study, γ-GT level was significantly higher in abortion compared with normal pregnancy. In a previous study, serum GGT level was found significantly lower during the second and third trimesters compared with non-pregnant women.[17] This decrease in GGT in late pregnancy can be explained by inhibition of hepatic synthesis of GGT by hormone secretion during pregnancy. Also, women suffering from viral hepatitis in early pregnancy was found with higher serum GGT level than women with viral hepatitis in late pregnancy. The same phenomenon was also found in women receiving oral contraceptives.[17]

Regarding serum TALP, PALP levels and PALP/TALP ratio in this study, it was significantly higher during the normal second trimester pregnancy compared with abortion at same trimester. As it is known, alkaline phosphatase (ALP) is present in many tissues of the body with particularly high levels in the liver, bone, placenta, intestine and kidney. These are referred to as isoenzymes and ALP is elevated in the serum in pregnancy, especially during the second and third trimesters.[1] The elevation in total ALP is a reflection of placental ALP entering the maternal blood, since human trophoblastic cells are rich in ALP. Measurement of serum ALP of placental origin is, therefore, of particular interest in the investigation of placental insufficiency. Also, extremely high serum TALP concentrations should arouse suspicion of bone, hepatic, endocrine, renal, or malignant diseases.[18]

It should be mentioned that the increase in the serum of total ALP during the second trimester of pregnancy was first recognized by Coryn in 1934,[19] whose observation has since been confirmed by other investigators.[20,21] The increase becomes apparent during the second trimester and continues throughout the third trimester reaching peak at term. The elevation in maternal TALP level usually disappears within a few weeks after delivery.[21]

CONCLUSION

Decrease serum TALP and PALP levels in 2nd trimester abortion women and the highly specificity and sensitivity of PALP/TALP ratio may help the gynecologist in predicting abortion especially when these enzymes were estimated at the middle of 2nd trimester (20 wks. of gestation).

Still, this decrease is not clarify enough, so a genetic study for total ALP and its placental isoenzyme (PALP) during second trimester may help in protect women from abortion.

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Table 1: Anthropometric, liver function tests and placental alkaline phosphatase activity in study groups

Anthropometric & Biochemical variables	Abortion at 2nd trimester women (n=41) (Patient group)	Pregnant at 2nd trimester (n=39) (Control Group)	P value
Age (years)	24.24± 1.1	24.3 ±1.02	NS
GA (wks.)	20.98± .35	20.94± .33	NS
Liver Function tests			
-Total Bilirubin (mg/dl)	0.69±0.03	0.62±0.01	0.039
-Direct Bilirubin (mg/dl)	0.17±0.001	0.16± 0.004	0.023
-GPT (IU/ml)	20.71±.58	24.69 ±0.28	0.0001
-GOT (IU/ml)	21.09± 053	23.58± 023	0.0001
GPT/GOT ratio	0.97±0.05	1.05±0.01	NS
γ-GT (IU/L)	24.64± 0.25	22.3± 0.11	0.0001
-TALP (ng/ml)	51.84 ±1.21	193.36± 1.12	0.0001
-Total Protein (g/dl)	7.13 ±1.22	6.95± 0.1	NS
-Albumin (g/dl)	3.88 ±0.01	3.64± 0.03	0.0001
-Globulin (g/dl)	3.08± 0.16	3.64± 0.13	0.025
-Alb/Glob ratio	1.38 ±0.06	1.05 ±0.03	0.0001
PALP (ng/ml)	1.77±0.06	12.36 ±0.21	0.0001
PALP/TALP ratio	0.04 ±0.001	0.06± 0.001	0.0001

Data were expressed as mean ± SE, NS= not significant, p value < 0.05 considered as statistically significant and < 0.01 highly significant.

Table 2: Correlations and linear regression equations between anthropometric and biochemical parameters in abortion women group at second trimester (n=41)

Correlations	r value	Linear regression equation (y)
Age + GA+ TSBn	0.403**	-0.1334x+24.213
	0.329*	0.0096x+0.3862
GPT/GOT ratio+ GPT+ GOT	0.704**	0.0578x+0.0479
	0.576**	- 0.052x+1.8453
TP + TSBn + Glob + Alb/Glob ratio	0.296**	0.0745x+0.1008
	0.337*	0.2653x+6.1321
	0.0346*	0.168x+2.5538
PALP + GA + DSBn + PALP/TALP ratio	0.355*	-0.0622x+3.0752
	0.294*	12.904x-0.401
	0.836**	0.0202x-0.0007

Note: r=correlation value, p value < 0.05 considered as statistically significant (*) and < 0.01 highly significant (**).

Table 3: Correlations and linear regression equations between biochemical parameters in group of normal pregnant women at second trimester (n=39)

Correlations	r value	Linear regression equation (y)
GPT + GOT + GPT/GOT ratio	0.419**	0.335x+15.30
	0.643**	0.027x+0.384
GOT +GPT/GOT ratio	0.424**	- 8.0801x+32.054
TALP + GA + DSBn + PALP/TALP ratio	0.405*	- 0.14x+48.03
	0.402*	-125.8x+213.4
	0.40*	-386x+218.0
Glob + TP + Alb/Glob ratio + PALP + PALP/TALP ratio	0.363*	0.278x+6.119
	0.939**	-3.692x+7.499
	0.366*	-0.237x+6.576
	0.346*	-0.003x+0.074

Note: r=correlation value, p value < 0.05 considered as statistically significant (*) and < 0.01 highly significant (**).

Table 4: Validity of the diabetic diagnostic tests in patients groupusing ratio positive = sensitivity / (1− specificity) equation

Test	Sensitivity (%)	Specificity (%)	Probability ratio of a positive test
PALP/TALP ratio	69.58	91.64	8.32
GPT/GOT ratio	66.16	87.63	5.35
Alb/Glob ratio	64.27	86.80	4.87

PALP=placental alkaline phosphatase, TALP= total alkaline phosphatase, GPT=glutamic pyruvic transaminase, GOT= glutamic oxalo acetic transaminase, Alb= albumin, and Glob= globulin.

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