

Histochemistry of Placental Alkaline Phosphatase in Preeclampsia

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Abstract

Objectives: Placental alkaline phosphatase (PALP) is synthesized in placenta and increases with gestational age. Alkaline phosphatase supports pregnancy and could play an essential role in nutrient supply and growth of the fetus. Preeclampsia is a systemic disorder which affects 5 to 7 percent of women worldwide and is a major cause for maternal and neonatal morbidity and mortality. As it has a major role in fetal growth, nutrition and defense mechanism study of alkaline phosphatase enzymatic activity becomes essential.

Methods: 50 normal and 50 preeclamptic placentae were collected immediately after delivery from Department of Obstetrics and Gynecology. Placentae were obtained from known preeclamptic consenting cases who had no history of hypertension before pregnancy or during first 20 weeks of gestation, who had consistently recorded systolic and diastolic blood pressure of 140 / 90 mm of Hg or above and proteinuria \geq 300mg / 24 hrs. Alkaline phosphatase activity was demonstrated by using modified Gomori's method.

Results: Intensity of PALP localization was stronger in preeclamptic placentae as compared to normotensive placentae.

Conclusion: Placental ischemia is evident in preeclampsia. Uteroplacental insufficiency leads to increased syncytial damage in preeclamptic placentae which may lead to abnormally high PALP activity and consequent increase in serum alkaline phosphatase.

Keywords: Preeclampsia, Pregnancy, Alkaline phosphatase, Placenta, Ischemia

1. Introduction

Placenta is a vital organ playing central role in pregnancy. It maintains pregnancy and promotes normal fetal development and serves as a major organ for transfer of essential elements between mother and fetus. [1]

Preeclampsia is a systemic disorder defined as development of hypertension and proteinuria after 20 weeks of gestation in previously normotensive woman. Preeclampsia affects 5 to 7 percent of women worldwide and is a major cause for maternal and neonatal morbidity and mortality. Preeclampsia is often associated with intrauterine growth restriction. [2]

Human alkaline phosphatase is found in higher concentrations in liver, bile duct, bone, intestines and placenta. [3] Alkaline phosphatase is one of the important enzymes secreted by placenta. Placental alkaline phosphatase (PALP) is polymorphic and heat stable enzyme and high levels of this enzyme is found in trophoblast of placenta. It is localized in apical and basal cells of syncytiotrophoblast

plasma membrane. [4] It is synthesized from placental syncytiotrophoblast from the twelfth week of pregnancy and is released into the maternal blood. In early pregnancy PALP activity is low. Measurable levels of PALP appear in maternal serum by the end of first trimester and increases progressively with gestational age and normally peaks at term. [3,5,6] It is said to be involved in nutrient transport from mother to fetus and also in transport of maternal IgG to the fetus.[7] It has a role in active transport of phosphates, absorption of nutrients and uptake mechanism through the plasma membrane.[8,9] Suggestions have been made that this enzyme is involved in transfer of glucose and fatty acids across the cell membrane.[10] Alkaline phosphatase is reported to be concerned with carbohydrate and phospholipid metabolism. It has been used as a biochemical marker in bone diseases and conditions such as prostatic carcinoma and myocardial infarction.[11,12] Thus PALP contributes to the maintenance of fetal health by being involved in defence from toxic substances and nutrient mobilization. The purpose

of this study is to find out the exact histochemical localization of alkaline phosphatase activity in preeclamptic placentae and its correlation with growth and development of the fetus.

2. Materials and Methods

Cross sectional study was conducted in Department of Anatomy of our medical college. Consecutive convenient sampling method was done. 50 normal and 50 preeclamptic placentae were collected immediately after delivery from Department of Obstetrics and Gynecology of our hospital. Institutional ethical committee clearance was taken. Written informed consent was obtained from all mothers participating in the study.

Samples were divided into two groups as Group A and Group B.

2.1 Group A: Control group (Normotensive)

Placentae were obtained from pregnant women who did not have any clinically detectable abnormalities. These women had normal blood pressure, no proteinuria and no oedema.

2.2 Group B: Study group (Preeclampsia)

Placentae were obtained from known preeclamptic cases that had no history of hypertension before pregnancy or during first 20 weeks of gestation, who had consistently recorded systolic and diastolic blood pressure of 140 / 90 mm of Hg or above and proteinuria ≥ 300 mg/24 hrs. Detailed menstrual and obstetric history and past history was obtained to exclude preexisting hypertension and other complications. Fetal weight, sex, any congenital anomaly and APGAR score at 1 and 5 minutes after delivery were recorded as parameters of fetal outcome. Serum alkaline phosphatase of both control and preeclamptic women was recorded to correlate with placental alkaline phosphatase activity.

Alkaline phosphatase activity was demonstrated by using modified Gomori’s method. Whole thickness tissue was cut out from central and peripheral part of placenta. Frozen sections of 10 micron thickness were taken from this fresh unfixated tissue. These were further stained by modified Gomori’s method at pH 9.100 villi were studied from each of central and peripheral section of placentae for distribution of enzymatic activity. Quantification of enzymatic activity was assessed visually and was classified based on the extent of distribution of enzymatic activity in the layers of villi from + to + + + + +.

3. Results

Table 1: Maternal parameters of control and study group

	Blood Pressure (mmHg)		Serum Alkaline phosphatase Mean ±SD (IU)
	Systolic	Diastolic	
Control group	118±5.42	78.68± 3.56	199.66 ± 19.50
Study group	156.36±14.98	98.84±6.06	294.46 ± 28.16

P value < 0.05 – Statistically significant

Table 2: Fetal parameters of control and study group

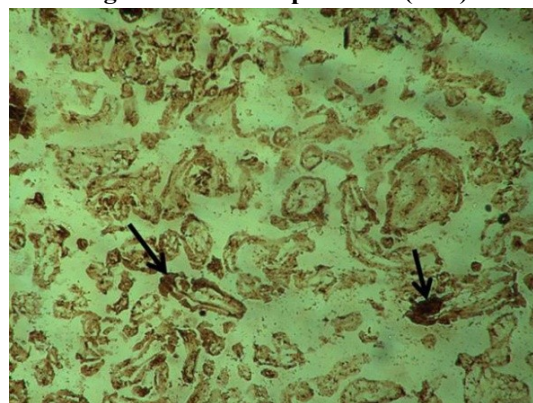
Fetal parameters	Control Group Mean ± SD	Study Group Mean ± SD
Birth weight (kg)	2.8±0.46	2.1± 0.39
APGAR score	8.9±0.76	6.5 ±0.95

P value < 0.05 – Statistically significant

3.1 Under low power

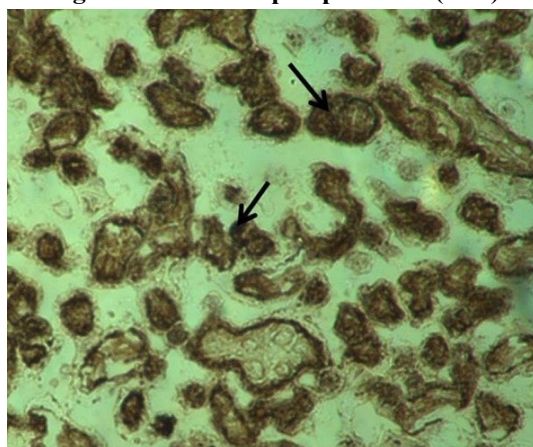
Preeclamptic placentae showed increased intensity of PALP activity as compared to control placentae. [Figure 1 and 2]

Figure 1: Control placenta. (10X)



Arrows showing PALP activity

Figure 2: Preeclamptic placenta. (10X)



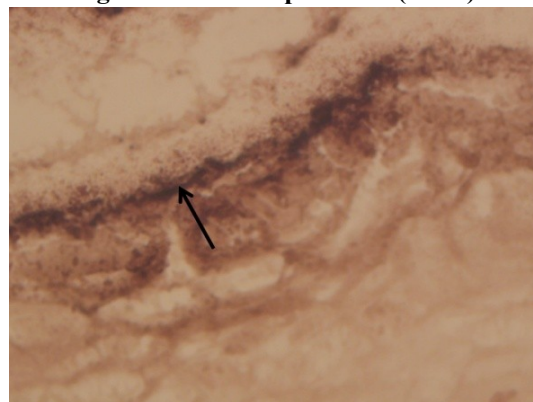
Arrows showing strong PALP activity

3.2 Under high power

3.2.1 Control Placentae

(+) : Out of 50 placentae majority of villi in central section of 14(28%) and peripheral section of 15(30%) placentae showed: [Figure 3]

Figure 3: Control placenta. (100X)



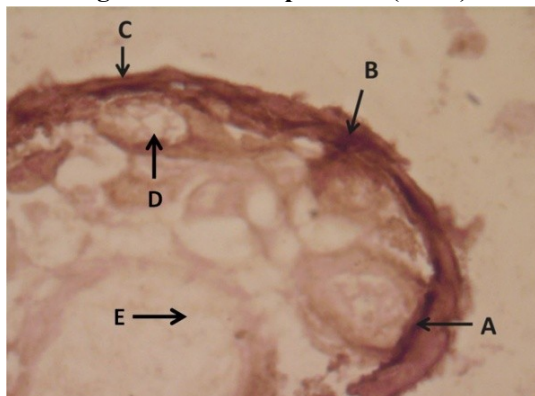
PALP activity was observed only on basal syncytial membrane.

PALP localisation was found only on the basal syncytial membrane.

Cytoplasm, microvillous surface of syncytiotrophoblast, cytotrophoblasts and connective tissue stroma did not show any localisation.

(+++): Out of 50 placentae majority of villi in central section of **36(72%)** and peripheral section of **35(70%)** placentae showed: [Figure 4]

Figure 4: Control placenta (100X)



- A- Strong PALP activity observed on basement membrane of syncytiotrophoblast.
- B, C -Moderate activity observed over cytoplasm and microvillous surface of syncytiotrophoblast.
- D- No PALP activity observed on cytotrophoblast
- E- No PALP activity observed on connective tissue stroma

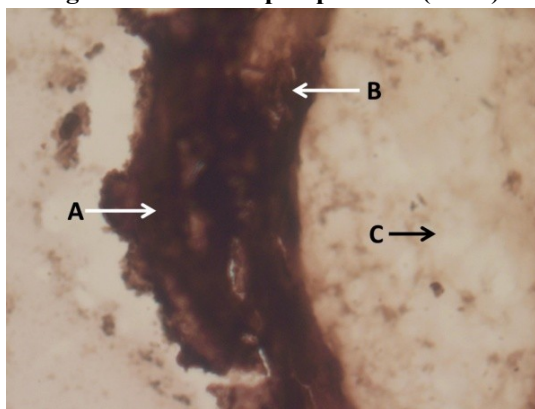
Strong PALP localisation was found on the basal syncytial membrane, slightly low intensity PALP localisation on was found on cytoplasm and microvillous surface of syncytiotrophoblast.

Cytotrophoblasts and connective tissue stroma did not show any localisation.

3.2.2 Preeclamptic Placentae:

(++++): Out of 50 placentae majority of villi in central section of **10(20%)** and peripheral section of **11(22%)** placentae showed: [Fig 5]

Figure 5: Preeclamptic placenta (100X)

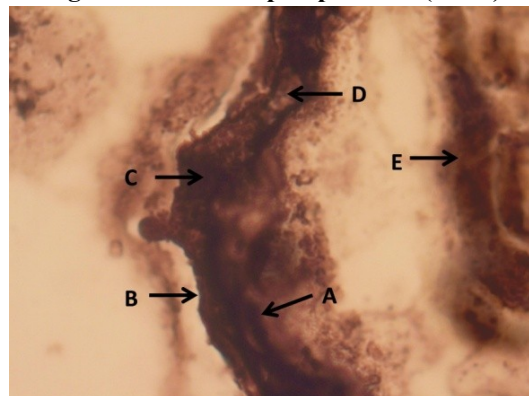


- A- PALP activity observed on syncytiotrophoblast
- B- PALP activity observed on cytotrophoblast
- C- Connective tissue stroma does not show PALP activity

High intensity of PALP localisation was found on the basal syncytial membrane, cytoplasm and microvillous surface of syncytiotrophoblast. Slightly low intensity of PALP activity was observed on cytotrophoblast cells. Connective tissue stroma did not show any localisation.

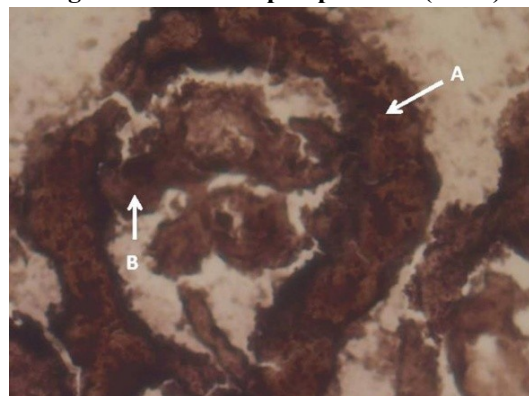
(+++++): Out of 50 placentae majority of villi in central section of **38(76%)** and peripheral section of **36(72%)** placentae showed: [Figure 6 and 7]

Figure 6: Preeclamptic placenta (100X)



- A- Intense PALP activity observed on syncytial basal membrane.
- B- Strong activity observed on syncytial microvillous surface
- C- Strong PALP activity observed in cytoplasm of syncytiotrophoblast
- D- PALP activity observed on cytotrophoblast
- E- PALP activity observed in connective tissue stroma

Figure 7: Preeclamptic placenta (100X)



- A.Strong PALP localisation observed in syncytiotrophoblast
- B.Strong PALP localisation observed in connective tissue stroma

High intensity of PALP localisation was found on the basal syncytial membrane, cytoplasm and microvillous surface of syncytiotrophoblast.

Slightly low intensity of PALP activity was observed on cytotrophoblast cells.

Connective tissue stroma also showed strong PALP localisation

(+++): **However**, majority of villi in central section of remaining **2(4%)** placentae and peripheral section of remaining **3(6%)** placentae showed PALP distribution similar to that of control placentae.

PALP localisation was observed on the basal syncytial membrane, cytoplasm and microvillous surface of syncytiotrophoblast.

Table 3: PALP localisation

PALP activity	Control placentae		Preeclamptic placentae		Inference
	Central section	Peripheral section	Central section	Peripheral section	
+	14(28%)	15(30%)	—	—	PALP activity observed only on basal syncytial membrane
+++	36(72%)	35(70%)	2(4%)	3(6%)	PALP activity observed on basal syncytial membrane, syncytial cytoplasm and microvillous surface of syncytiotrophoblast
++++	—	—	10(20%)	11(22%)	PALP activity observed on basal syncytial membrane, cytoplasm, microvillous surface of syncytiotrophoblast and cytotrophoblast cells
+++++	—	—	38(76%)	36(72%)	PALP activity observed on basal syncytial membrane, cytoplasm, microvillous surface of syncytiotrophoblast ,cytotrophoblast cells and connective tissue stroma

4. Discussion

PALP has been extensively studied by various workers. Elevated PALP levels may indicate premature delivery. Various studies have shown that alkaline phosphatase levels in maternal serum can be used as a marker for idiopathic preterm delivery. [4] In several studies this enzyme has been used as a biochemical marker of metabolic bone disease in neonates. [13]

In present study it was found that PALP activity was more intense in preeclamptic placentae as compared to control placentae. This finding was similar to Mangal *et al*, Jeacock *et al*, Curzen P and Dempsey *et al* [8,11,14,15] who found increased amount of PALP localisation in preeclamptic placentae as compared to control placentae. In present study majority of villi in control placentae showed PALP activity localized over the basal membrane of syncytiotrophoblast, moderate activity was seen on the microvillous surface and cytoplasm of syncytiotrophoblast. PALP activity on basal syncytial membrane has been reported by Matsubara *et al* [9] and Lister *et al*. [16] This could be because placental alkaline phosphatase is produced from the basal membrane of syncytiotrophoblast.[17] Matsubara *et al*, Kameya *et al* and Jones *et al* [9,18,19] have earlier reported PALP activity on cytoplasm and microvillous surface of syncytiotrophoblast.

Preeclamptic placentae showed a very strong localization of PALP in both central and peripheral sections suggesting increase in the placental alkaline phosphatase activity. In majority of villi in preeclamptic placentae very strong PALP activity was seen on basal membrane, apical microvillous surface of syncytiotrophoblast, cytoplasm of syncytiotrophoblast, cytotrophoblast and connective tissue stroma. This observation is in accordance with previous workers like Mangal *et al*[8] and Curzen P[14] who observed strong PALP activity in syncytiotrophoblast of preeclamptic placentae as compared to control placentae. Dempsey *et al* [15] reported PALP activity in connective tissue stroma of preeclamptic placentae. However the findings in the present study differ from previous workers like Sammak *et al*, Boronkai *et al* and Francis *et al* [17,20,21] who observed decreased alkaline phosphatase activity in preeclamptic placentae as compared to control placentae.

Placental ischemia is evident in preeclampsia. There is decrease in synthetic activity of trophoblast and cellular

respiration is also probably depressed. [22] PALP appears to be moderately resistant to hypoxia. Preeclamptic placentae showed considerable increase in lysosomal activity. This is presumably a response to placental ischemia, which leads to alteration in the pH of trophoblast and stimulates lysosomal activity. Thus syncytial damage is apparent in preeclamptic placentae. Syncytial damage and destruction in preeclampsia leads to release of PALP from vesicles into cytoplasm.[19] Increased amount of placental alkaline phosphatase enzyme was found in toxemic placentae at 34-37 weeks. Ischemic changes in trophoblast during this period could be the reason for marked rise in PALP activity in toxemic preeclampsia. Premature placental ageing in toxemia is characterized histochemically by accumulation of PALP in syncytiotrophoblast.[11]

The other parameter recorded in present study was serum alkaline phosphatase. Serum alkaline phosphatase in pregnancy is mainly of placental origin.[5,21,23,24] Placental alkaline phosphatase activity is localized to the external surface of syncytial villi and are in close relation with maternal circulation. This suggests that the enzyme is released from the trophoblast and major proportion of it enters the maternal blood.[16,25] Thus, its increased level in maternal serum might be due to increased PALP. In present study it was observed that serum alkaline phosphatase levels were significantly high in preeclamptic cases as compared to normotensive controls. This finding is in accordance to previous workers like Curzen P[26], Mangal *et al*[8] and Kapoor *et al*. [27] Women with high maternal blood pressure had higher levels of serum alkaline phosphatase and increased intensity of PALP localization in placentae. Similar findings were reported by Mangal *et al*[8] and Curzen P.[14] Abnormally high serum alkaline phosphatase levels represent placental damage and failing placental function.[14,27,28] Placental ischemia, necrosis, damage of chorionic villi and infarction can increase the levels of PALP in maternal serum.[4,8] PALP shows rising levels in maternal serum in second and third trimester of pregnancy. This coincides with the period of fetal osteogenesis and calcification of fetal skeleton. Mobilization of calcium from maternal system for fetal calcification process is facilitated by PALP. This confirms placental origin of alkaline phosphatase in maternal serum.[8,26]

Rate of secretion and production of alkaline phosphatase has been observed to correlate strongly with the increasing nutritional demands of growing fetus.[29] PALP is said to be involved in nutrient transport from mother to fetus.[3]

In present study it was observed that mean fetal birth weight and APGAR score in preeclamptic cases was lower than those of normotensive controls. This finding is similar to Mangal *et al* [8]; however they found no correlation of serum alkaline phosphatase and fetal birth weight. Many workers like Myatt *et al* [30], Soma *et al* [31] and Eskild *et al* [32] suggested that placental insufficiency and impaired placental function in hypertensive pregnancy leads to low fetal birth weight. There is some evidence that compensatory changes which limit the effects of ischemic damage are brought into play in preeclampsia.[22] Since PALP has major role in transport and energy production increased PALP activity in preeclamptic placentae could be a compensatory mechanism to provide nutrition to the fetus in ischemic conditions.

5. Conclusion

In present study it can be concluded that syncytial damage due to placental ischemia may lead to abnormally high placental and consequent increase in serum and alkaline phosphatase. Ischemic placental damage affects the transport of important materials between mother and fetus eventually leading to poor nutrition of fetus and low fetal birth weight. Another probable reason could also be that increase in alkaline phosphatase is to compensate for lack of nutrients supplied to the fetus due to placental ischemia as PALP has a major role in nutrient supply and maintenance of fetal health. PALP could be used as a marker for detecting ongoing placental damage and IUGR and further damage could be arrested.

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