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HISTOLOGICAL AND HISTOCHEMICAL EVALUATION OF NORMOTENSIVE AND PREECLAMPTIC PLACENTAS

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Abstract: *The placenta plays a role in the pathophysiology of preeclampsia. Preeclampsia is more common in multifetal pregnancies than singleton pregnancies. In this study, we aimed to investigate the histopathology of normotensive and preeclamptic placentas and the localization of alkaline phosphatase activity. In our study, 10 normotensive and 10 preeclamptic, totally 20 placentas were obtained.Paraffin sections were stained with Hematoxylin-Eosin, Masson trichrome and PAS for histopathological examination. Remaining sections were then stained via Gomori's method and micrographed under light microscope. Sections of the control group observed normal histologically structure. The alkaline phosphatase reaction was evident in the inner and outer membranes of the syncytiotrophoblasts. There was a significant increase in the number of syncytial knots, terminal villi and syncytial bridges in the preeclamptic placenta sections. Marked thickening of the trophoblast basal membranes were observed. Alkaline phosphatase reaction in preeclampsia group: The localization of alkaline phosphatase in the inner and outer membranes of the syncytiotrophoblasts was reduced. In placentas of preeclampsia group, a significant increase in syncytial knot, syncytial bridge were observed. The level of alkaline phosphatase enzyme in preeclamptic placentas was found to be lower compared to normotensive placenta.*

Key words: *Placenta, Preeclampsia, Alkaline phosphatase, Light microscope.*

1. Introduction

The placenta plays a role in the pathophysiology of preeclampsia. Preeclampsia is more common in multifetal pregnancies than singleton pregnancies [1]. In the preeclampsia although its starting point is not known, reduced placental perfusion and the destruction of the placental tissue which leads to maternal vascular endothelium dysfunction are said to be responsible mechanism of preeclampsia [2].

Normally, the alkaline phosphatase enzyme (PALP) is produced from the basal membrane of the microvilli on syncytiotrophoblasts and their surfaces. This enzyme is produced in small amounts

during the first and second trimester, increases in the third trimester and reaches the maximum amount in the full-term placenta [3].

This enzyme is responsible for the active transport of phosphate [4], the transfer of maternal IgG to the fetus [5-6], the absorption of nutrients [7] and the growth and development of the fetus. The placenta synthesizes PALP in the second and third trimester of pregnancy and the PALP concentration slowly increases till the term. Reduced serum PALP level in pregnant women may be related to intrauterine growth retardation [8-9].

In this study, we aimed to investigate the histopathology of normotensive and preeclamptic placentas and the localization of alkaline phosphatase activity

2. Material and Methods

In our study, 10 normotensive and 10 preeclamptic, totally 20 placentas were obtained. 1x1x1 cm^3 tissues were dissected and fixed in 10% neutral formalin. 4 µm paraffin sections were stained with Hematoxylin-Eosin (H&E), Masson trichrome and periodic acid Schiff (PAS) for histopathological examination. Remaining sections were taken to positive-charged slides and incubated at room temperature for 1 hour. Sections were then stained via Gomori's method and micrographed taken under light microscope.

3. Results

3.1. Control group results

3.1.1. H&E and PAS staining results of control group

Syncytiotrophoblast layer, villus stroma, fetal vascular structures, intervillous space and Hofbauer cells and trophoblast basal membranes were histologically observed normal structure in placenta sections of the control group (Figure 1).

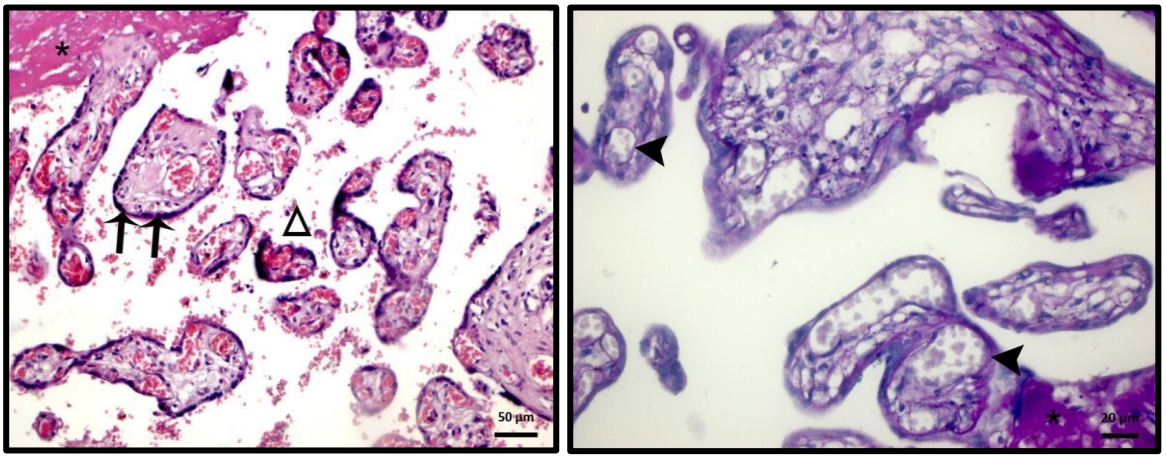

Figure 1: Placental sections of the control group. **a)** Syncytiotrophoblast cells (double arrow), perivillous fibrin accumulation (asterisk) and intervillous space (empty triangle) are seen (H.E, Bar:50 µm). **b)** Trophoblastic basement membranes in normal thickness (arrowhead) and perivillous fibrin accumulation (asterisk), (PAS, Bar: 20 µm).

3.1.2. Control group alkaline phosphatase reaction

Alkaline phosphatase reaction was strongly observed in sections obtained from full-term placentas without a history of preeclampsia or any pregnancy complications. When we examined the

placenta sections of the control group with a large magnification, the alkaline phosphatase reaction was evident in the inner and outer membranes of the syncytiotrophoblasts. While the reaction was continuously monitored on the outer membrane, it was non-continuous on the inner membrane.
On the other hand, villous stroma and maternal decidua showed a moderate alkaline phosphatase reaction. While the alkaline phosphatase reaction was negative in cytotrophoblasts, alkaline phosphatase reaction was found to be poor in villous stroma and maternal decidua of full-term preeclamptic placentas (Figure 2).

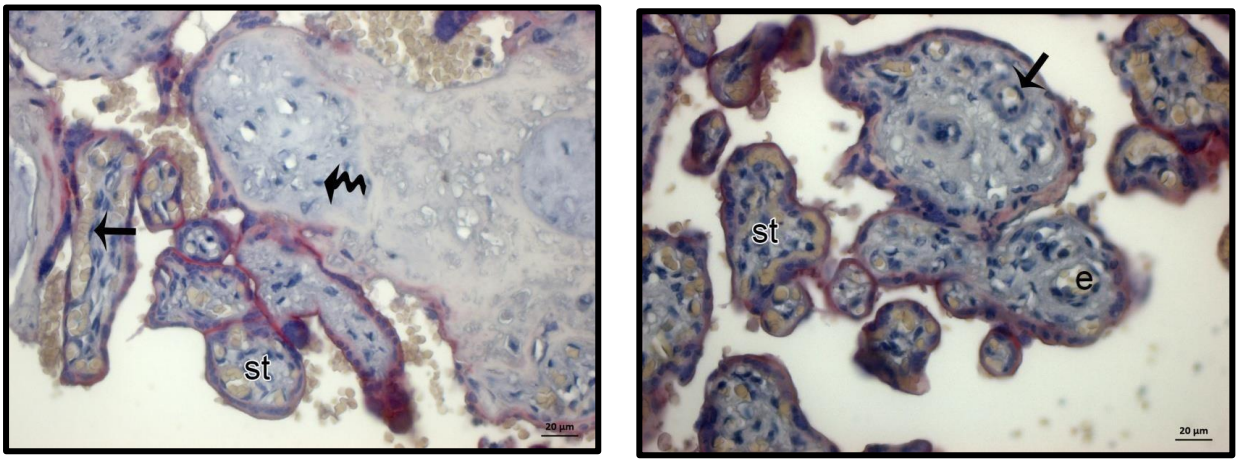

Figure 2: Placental sections of the control group. **a)** Negative alkaline phosphatase enzymatic reaction in villus stroma (st), capillary endothelium (thin arrow) and decidua cells (convoluted arrow) but positive reaction in syncytiotrophoblasts (ALP, Bar: 20 µm). **b)** Negative alkaline phosphatase enzymatic reaction and fetal erythrocytes (e) are observed in placental villus stroma (st) and capillary endothelium (thin arrow) (ALP, Bar: 20 µm).

3.2. Placenta results in preeclampsia group

3.2.1. H&E and PAS staining results of preeclampsia

There was a significant increase in the number of syncytial knots, terminal villus and syncytial bridges in the placenta sections.

In addition, dilatation, proliferation and congestion were seen in the capillaries of fetal terminal villi. A labyrinth-like appearance was observed in preeclamptic placenta sections due to syncytial knots and bridges between villi Marked thickening of the vasculosyncytial and trophoblast basal membranes were observed as well as a dense fibrinoid increase in the perivillous and intervillous area (Figure 3).

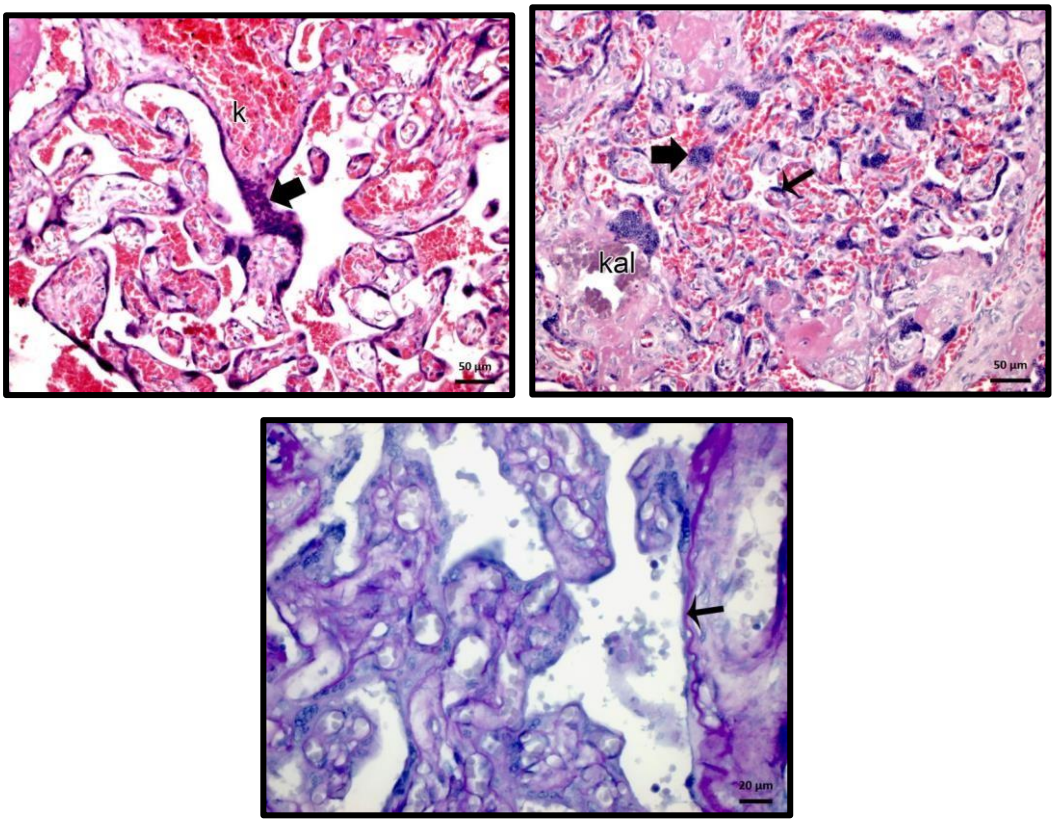

Figure-3: Placenta sections of the preeclampsia group. **a)** Dilatation in the fetal capillaries (k) intense increase in syncytial knots and syncytial bridges (thick arrow), (H.E, Bar: 50 µm). **b)** Focal calcification focus (kal) with intense syncytial bridges (thick arrow) and syncytial knots (thin arrow) (H.E, Bar:50 µm). **c)** Thickening and fibrinoid accumulation in vasculosyncytial membranes (arrowhead) (PAS, Bar:20 µm).

3.2.2. Alkaline phosphatase reaction in preeclampsia group

In the examination of placenta sections of preeclampsia patients; The localization of alkaline phosphatase in the inner and outer membranes of the syncytiotrophoblasts was reduced.

In different villi, even in some of the cells in the same villi, presence of both weak and strong enzymatic activity leads to a heterogeneous general appearance in placenta (Figure 4).

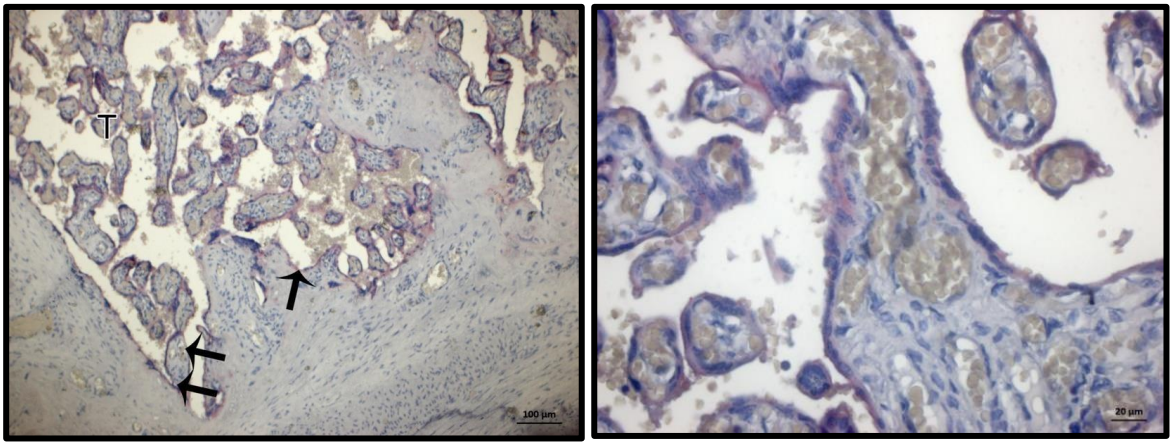

Figure-4: Placenta sections of preeclampsia group. a) Alkaline phosphatase reaction in placenta basal plate, cytotrophoblastic shell (thin arrow), anchoring villi (double arrow) and terminal villi (t) was observed at lower density than control sections (ALP, Bar: 100 µm). b) Higher magnification of the placenta section (ALP, Bar: 20 µm).

4. Discussion

As stated in the results, control sections of placenta showed a positive alkaline phosphatase reaction in all villi, especially in syncytiotrophoblast cells. In the literature, alkaline phosphatase activity was observed in Hofbauer cells, but only one study stated the reaction was very different [9]. In our study, we did not observe such finding. Although our results of enzyme placement were consistent with the findings of some researchers [5-8]; it is not supported by some other articles. Wielenga et al [9] observed negative enzymatic results in the nuclei of vascular and syncytial cells. This result may depend on the incubation time of the tissue and the methods applied.

Our findings regarding the distribution of the enzyme alkaline phosphatase in full-term placentas obtained from pregnant women during the control group period without a history of preeclampsia or other maternal complications were similar to those reported by other workers [10]. Alkaline phosphatase is an important enzyme for trophoblastic transfer, so it is very abundant in the full-term placenta [11]. Placental alkaline phosphatase (PALP) has been the subject of many studies because it plays an important role in the transport mechanism. We observed that the PALP was localized in the outer and inner layers of the syncytiotrophoblasts, and villi stroma of placentas of the control group (normotensive) were negatively stained. Some researchers found that PALP residues of control group placentas continued uninterruptedly on the brush edge of the outer membrane of the syncytiotrophoblasts, however they were noncontinuous in the inner membrane. Fetal stroma, blood vessels and cytotrophoblast showed negative staining. Our study confirms the reported observations of PALP in the placenta. In our study, PALP activity was found to be more intense in the control placenta than in the preeclamptic placenta. This finding is in contrast to the studies of Mangal et al., Jeacock et al., Curzen P and Dempsey et al [12-15]. The very strong PALP activity found in most of the villi of control placenta was seen on the basal membrane, the apical surface and cytoplasm of syncytiotrophoblast microvilli. This finding was in contrast to Mangal et al [16] and Curzen P et al [14] findings, which is strong PALP activity in the preeclamptic placenta compared to control placenta.

Dempsey et al. [15] reported that the preeclamptic placenta had PALP activity in the connective tissue stroma, but we did not find any such findings in our study.

In our study of histological examination of the control group placentas; villus trophoblast layer, villus stroma, fetal vascular structures were seen in normal appearance, while there was an increase in fetal volume, fetal capillary count, fibrinoid accumulation, Hofbauer cells and atrophic villi in preeclamptic placentas (Figure-3-4). These findings in preeclamptic placentas are consistent with other studies investigating placental histopathology in preeclampsia [17-20].

In placentas of preeclampsia group, a significant increase in syncytial knot, syncytial bridges, atrophic villus and perivillous fibrin accumulation and a significant thickening of the trophoblast basal membrane were observed. The level of alkaline phosphatase enzyme in preeclamptic placentas were found to be lower compared to normotensive placenta.

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