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AN IMMUNOPATHOLOGICAL STUDY ON THE PLACENTA IN PRE-ECLAMPSIA

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INDEXING WORDS

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SYNOPSIS

An investigation was done to clarify the pathological background of pre-eclampsia. Thirty four placentae were studied immunopathologically in comparison to control cases. The results showed slightly positivestains of hCG containing alpha- and beta-subunits in syncytiocytotrophoblastic layers especially in severe pre-eclampsia cases. The placentae in mild pre-eclampsia showed only weakly and partly positive by those stains. Other control cases were negative except earlier abortion ones. No any positive stains of hCG series could be seen in two placentas from mothers with spontaneous hypertension. Stains by hPL and SP 1 were all positive and increased gradually in their staining pattern and degree from early pregnancy toward term.

It supports the findings that maternal serum hCG series increased slightly in pre-eclampsia and that those changes might occur before clinical onset of pre-eclampsia. Then, the placenta has an important role in developing pre-eclampsia.

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INTRODUCTION

In a recent review it was obviously stated that the placenta occupies a unique position among the endocrine glands and plays a central role in the endocrinology of pregnancy.[13] HCG and hPL are two important peptide hormones produced by the placenta that play an essential role in maintaining pregnancy. Many studies concluded that hCG and hPL are specific products of villous trophoblasts of the placenta, and it is believed that syncytiocytotrophoblasts are main areas of their production, as likely also for alpha and beta hCG.[5,6,8,9,10]

In pre-eclampsia placenta should have very important roles even though clear etiologicai factors are still disputable, as it is realized that in pre-eclampsia when the baby has already been delivered, the symptomes will disappear just right after the placenta released. Pre-eclampsia can be even developed in the condition without fetus i.e. hydatidiform mole.[4]

It had been recognized that in pre-eclampsia the maternal serum level of hCG especially in beta hCG concentration is significantly higher than that in normal pregnancy. As most of pre-eclampsia mothers develop clinically in the third trimester, it is possible to detect any changes pathologically in their placenta and to examine hCG changes immunoenzymatically, which may be closely correlated to the pathogenetic background of the pre-eclampsia.

MATERIAL AND METHODS

Examined placentas were obtained from the departments of obstetrics gynecology, Gadja Mada university, faculty of medicine, and Kobe university school of medicine (Table 1). Those placentas obtained were fixed in 10% buffered formalin, and processed routinely in parafin blocks, then cut in 5 micra thick serial sections. Histological sections were stained by HE, PAS, Azan, and Elastica Van Gieson stainings, respectively. Immunoenzymatical stainings were used by PAP (peroxidase anti-peroxidase) methods. We used monoclonal antibodies against the free alpha hCG, beta hCG, and total hCG. HPL (human placental lactogen) for positive control as hPL is produced by syncytiotrophoblasts steadily from the first trimester of pregnancy until delivery. AFP was negative

control as AFP is not produced by the placenta. Negative controls were also prepared by either neglecting the primary antibody or relapsing the primary antibody with non-immune serum. Positive control tissues for hCG group staining were prophoblastic tissue obtained from abortion or tubal pregnancy, and negative control tissues for it were trophoblastic tissues obtained from normal term pregnancy. Staining results were divided to semiquantitative grading as negative, weakly positive, moderately positive, and strongly positive, by brownish red colour of positive staining.

Table I Cases of examined placentas.

Case	Number
Abortion(6-9 gestational weeks)	4
Prematurity	1
Normal term pregnancy	10
Essential hypertension	2
Mild pre-eclampsia	12
Severe pre-eclampsia	22
Total	51

RESULTS

Placenta of abortion in 9 gestational weeks showed immature lining of chorionic villi. They were lined by thick syncytiocytotrophoblastic layers, with thick basement membrane by PAS stain (Fig. 1A). Syncytiovascular membrane can't be found in chorionic villi. Capillary vessels in villi contained a few hemocytoblasts. Thick walled arteries were found in the stem villi. However, vascular changes not so marked by Elastica Van Gieson stain. Mild fibrosis in the stem villi was found by Azan stain. Immunohistoenzymatically, hCG, alpha and beta hCG were respectively positive, markedly in outer syncytial and moderately in inner cytotrophoblastic cells (Fig. 1B). AFP stain was completely negative. HPL and SP 1 were both positive but not so strong in syncytial layer (Fig. 2A and B).

The placenta of control term pregnancy revealed good syncytiovascular membranes and knot formation, occasionally fibrin or calcium deposit around chorionic villi. The basement membrane

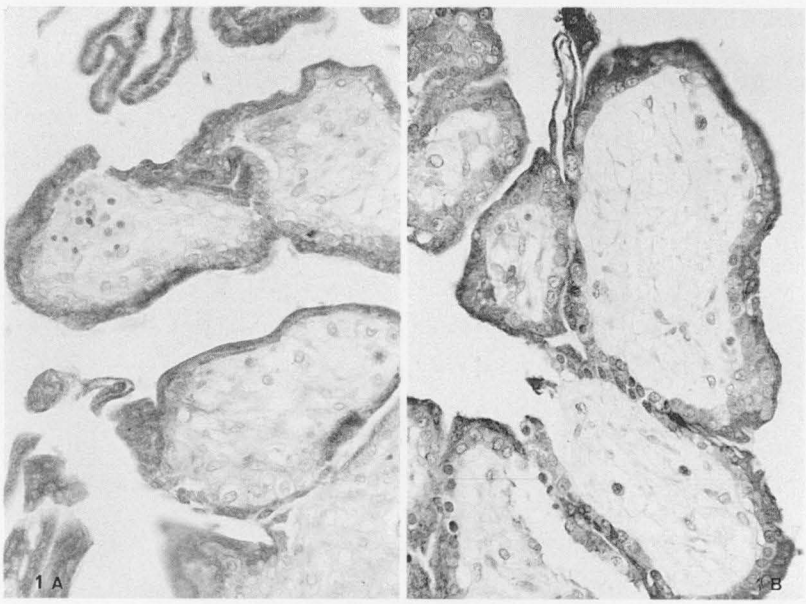

Fig. 1 Abortioned placenta of 9 gestational weeks: A, two cell layers of lining, PAS, x 200; B, markedly positive in outer syncytial and mildly in inner cytotrophoblasts, beta hCG, x 200.

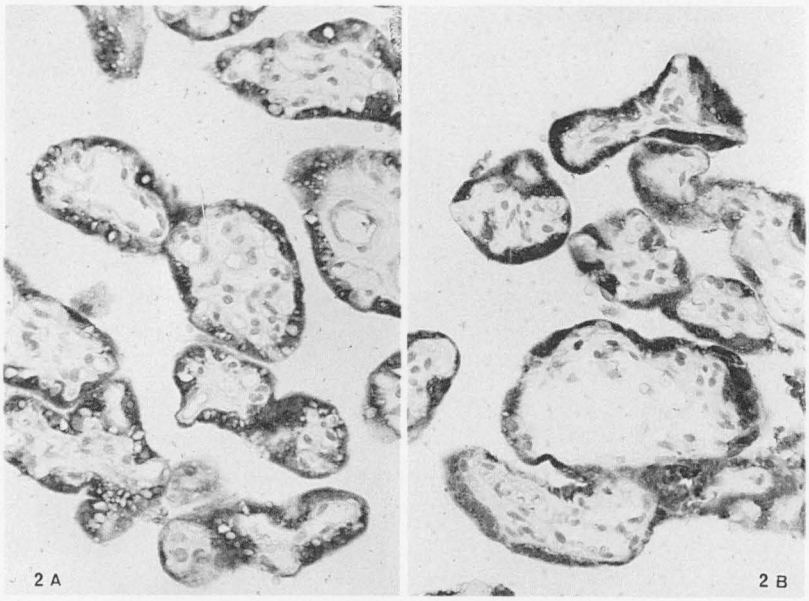

Fig. 2 Abortioned placenta of 9 gestational weeks: A, positive lining layers by hPL, x 130; B, positive lining layers by SP 1, x 130.

between villous epithelium and stroma was thin. The inner cytotrophoblasts were scarcely seen beneath the surface syncytial cells. Capillaries in chorionic villi were congestive. The stem villi had congestive veins and slightly sclerotic arteries, but no atherosis or inflammatory cells. No fibrosis could be seen in the stroma of villi or stemvilli. HCG series and AFP were both negative. HPL and SP 1 were positive clearly along the syncytial membrane.

The placenta from the mothers with spontaneous hypertension revealed almost same figures seen in control term ones. Capillary congestion and stromal edema were prominent in chorionic villi. The syncytial layer was thin with good vasculosyncytial membrane. The basement membrane was thin by PAS stain (Fig. 3A). HCG, alpha- and beta-hCG, and AFP were respectively negative (Fig. 3B). HPL and SP 1 were positive in the syncytial layer (Fig. 4A and B). The stem villi had sclerotic arteries with narrow lumina by Elastica Van Gieson stain.

The placenta obtained from mothers showing mild pre-eclampsia revealed capillary congestion in chorionic villi. Only a few cytotrophoblasts were persistent beneath the surface syncytial lining. Vasculosyncytial membrane were almost developed, but partly not developed with thick basement membrane by PAS stain (Fig. 5A) Syncytial knots and calcification, fibrin deposit were noted with infarcted foci. Stem villi had slightly thick arteries by Elastica Van Gieson stain. HCG, alpha- and beta-hCG were slightly and partly positive in syncytial cells (Fig. 5B). But AFP was completely negative. HPL and SP 1 were both positive in surface syncytial cells (Fig. 6A and B).

In severe pre-eclampsia cases, the chorionic villi were often lined by two cell layers of syncytiocytotrophoblastic layers, with thick basement membrane. Vasculosyncytial membrane was often disturbed even though there were many syncytial knots formations (Fig. 7A). HCG, alpha-, and especially beta-hCG were positive but not so strong. The surface syncytial cells were moderately positive but frequently not in succession aroung the villi. The inner cytotrophoblasts were weakly positive (Fig. 7B). AFP was negative anywhere of villi. HPL and SP 1 were both positive in the chorionic lining cells (Fig. 8A and B). The arteries in the stem villi were thick with narrow spaces. The veins were congestive and

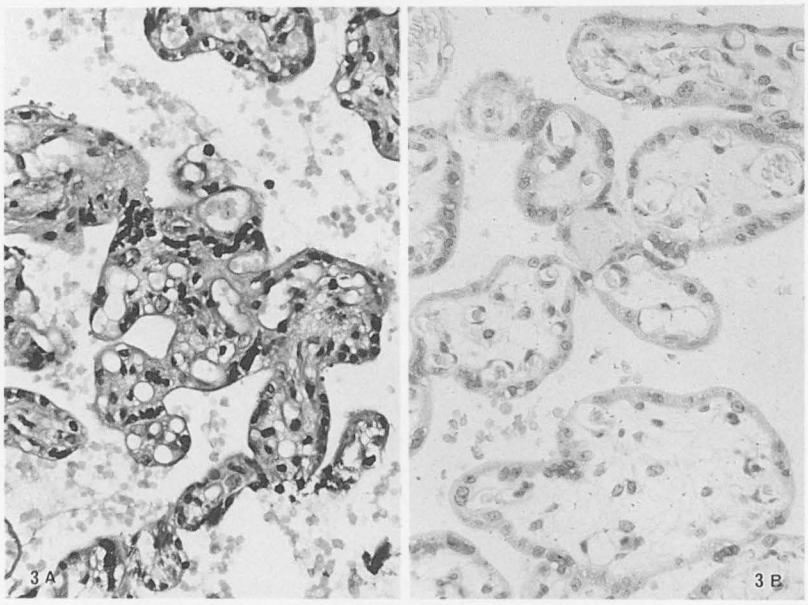

Fig. 3 Placenta obtained from a mother with essential hypertension: A, cappillary congestion and good vasculosyncytial membrane, PAS, x 100; B, completely negative stain by hCG, x 130.

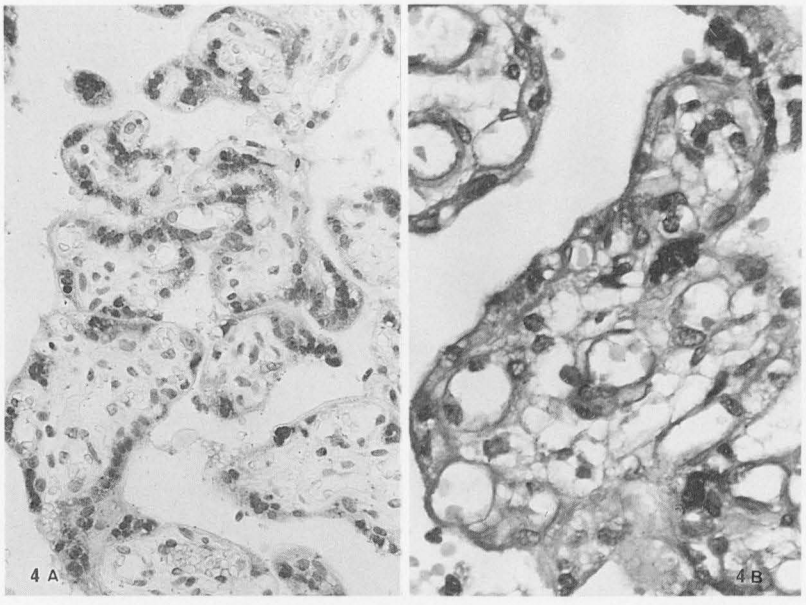

Fig. 4 Placenta obtained from a mother with essential hypertension: A, positive stain by hPL, x 130; B, positive stain by SP 1, x 130.

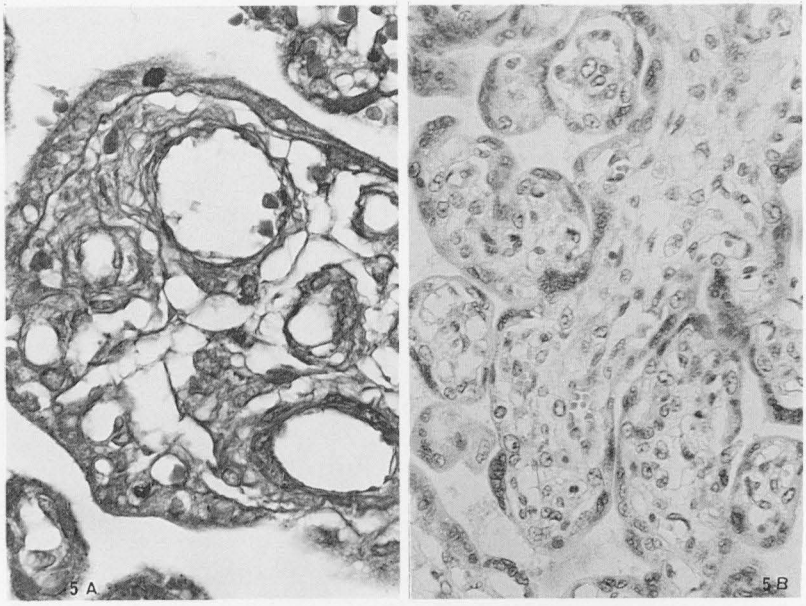

Fig. 5 Placenta of mild pre-ecplampsia: A, thick basement membrane, PAS, x 200; B, negative stain by beta hCG, x 130.

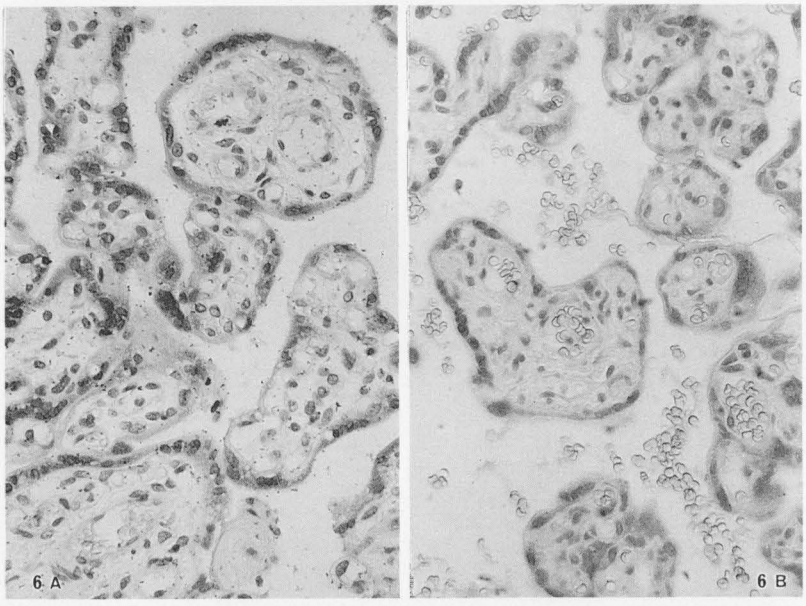

Fig. 6 Placenta of mild pre-eclampsia: A, positive stain by hPL, x 130; B, positive stain by SP 1, x 130.

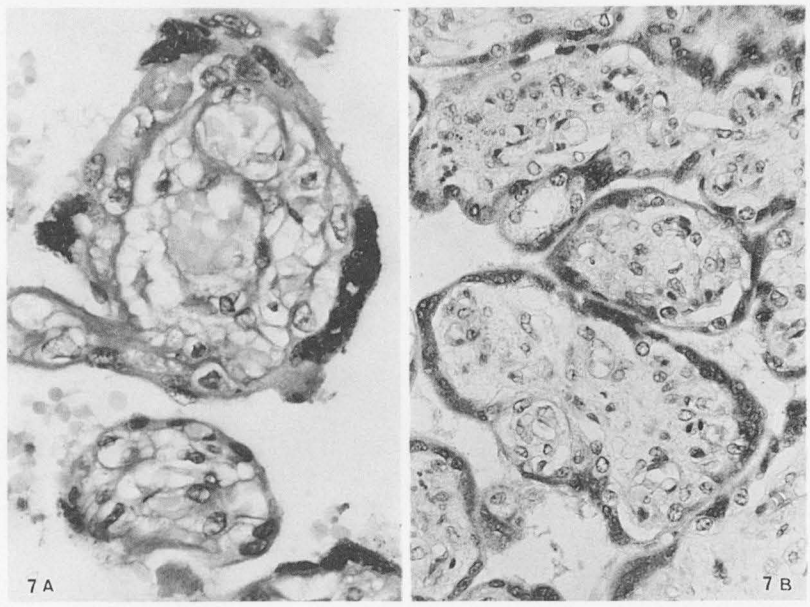

Fig. 7 Placenta of severe pre-ecplampsia: A, thick basement membrane and disturbed syncytiovascular membrane with knot formation, PAS, x 200; B, moderately positive stain by beta hCG, x 130.

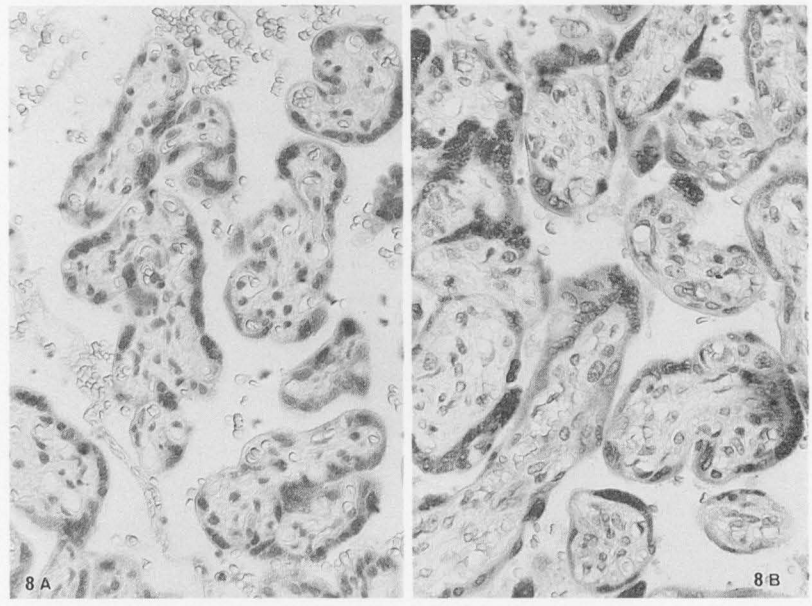

Fig. 8 Placenta of severe pre-eclampsia: A, positive stain by hPL, x 130; B, positive stain by SP 1, x 130.

dilatated in general. A few arteries in deeper decidual areas showed atherosis. Decidual change was various in cases, degenerative, partly inflammatory or intact.

DISCUSSION

The result showed that in pre-eclampsia there are real changes in the placental tissue both macroscopically or microscopically. Formerly, infarcted area in placenta was thought to be a specific sign for pre-eclampsia, but now it is still uncertain and disputable. And the microscopical findings in HE, PAS, and Van Gieson staining showed that they are in accordance with the former investigation reports. Quite specific changes such as thickening trophoblastic basement membrane and proliferation of cytotrophoblast can be found, but still can not be assessed whether these changes produce pre-eclampsia or as a result of pre-eclampsia.

In immunohistochemical staining for AFP all specimens were negative, as it is already known that AFP is not produced by the placenta. In general the reaction of abortion placenta to the immunohistochemical staining is characteristic, i.e., strongly positive for hCG and hCG-beta as their production reach their peak at the first trimester; weakly positive for hCG-alpha, hPL, and SP 1 as the production is low in the first trimester.[2,13)]

In term placenta there was no differences staining result between placenta from IUFD, essential hypertension, or normal term pregnancy. They have same pattern, i.e., negative hor hCG, hCG-beta as their production in syncytiotrophoblast falls to undetectable level,[3)] and this agrees with prior preliminary study by Utoro et al.[14)] In 1984 Beck et al.[1)] found that their investigation didn't agree with this finding, they report that they had been able to stain weakly positive for hCG and hCG-beta. Even though it could support our findings because Beck used the term absolutely weak positive that could be interpreted as practically negative reaction to hCG and hCG-beta staining. The other same pattern of staining result was moderately positive for anti hCG-alpha serum as this sub-unit is produced unit the end of pregnancy;[2,13)] and strongly positive reaction for hPL and SP 1 staining as their production reach their peak in the third trimester.

In placenta of pre-eclampsia there were no differences in

immunohistochemical reaction between mild and severe pre-eclampsia. The pattern was the same as weakly positive for hCG and hCG-beta staining, weak to moderately positive for hCG-alpha in concert with its steady production in trophoblastic tissue,[13] and moderately to strongly positive for HPL and SP 1 imply that there is no differences with normal term placenta in accordance with increasing production of hPL and SP 1 toward term, and even no relevancy with pre-eclampsia.[12,15]

All the positive reaction occured in syncytial lining except for hCG-alpha staining that some time also occurred in the cytoplasm of cytotrophoblast which are found under the syncytial lining, imply that there is proliferation of cytotrophoblast in term placenta of pre-eclampsia[7] that is not found in normal pregnancy.

The positive reaction to hCG and hCG-beta in term placenta of pre-eclampsia are quite interesting finding as the reaction in normal term placenta is negative, and this is in accordance with investigation by Crosignani et al.[4] that found increasing hCG serum concentration in mother with severe pre-eclampsia, and by Said et al.[11] that found increasing maternal serum level of hCG-beta concentration in pre-eclampsia. These facts imply that there are changes in the activity of the syncytiotrophoblastic cytoplasm that cause increasing maternal serum level of hCG and hCG-beta concentration pre-eclampsia.

Another interesting finding is negative reaction to hCG and hCG-beta in placenta of mother with essential hypertension. We examined only two cases that could be valid enough to be used comparison. Even though for the time being can be concluded that placenta should have central role in developing pre-eclampsia. This is according to the fact that in two kinds of hypertensive disorders in pregnant women, changes can only be found in placenta of pre-eclampsia, not in essential hypertension.

Apparently we can now answer the qeustion whether those above changes found in placenta produce pre-eclampsia or are produced by pre-eclampsia. With a little hesitation the answer is: the changes found in placenta produce pre-eclampsia. To be sure we could not find out in literature to date the increased hCG titer in maternal serum in cases of essential hypertension.

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