Immunohistochemical Demonstration of Intermediate Trophoblasts for Differential Diagnosis of Uterine and Ectopic Pregnancy

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Objective: **The objective in the study was to detect the intermediate trophoblastic cells and to differentiate the abortion from ectopic pregnancy by using immunohistochemical methods.**

Method: **Four groups of patients were included in the study, and the archival materials of the patients were used for immunohistochemistry. Group 1 comprised of 10 patients who had miscarriages between the 5th and 12th weeks of gestation. There were 5 non pregnant patients in group 2. In group 3, there were 64 patients who had ectopic pregnancy between the 5th and 12th weeks of gestation. Group 4 comprised of 50 patients who were clinically suspected of miscarriage without chorionic villi, and cyto and syncytiotrophoblast.**

Results: **All trophoblastic cells of chorionic villi showed strong positivity for sitokeratin in group 1 though this was not the condition in group 2 and 3. In group 4, however, 35 of 50 patients had intermediate trophoblast cells as detected by cytokeratin staining.**

Conclusion: **The use of these hormone markers in endometrial specimens increases precision in the diagnosis of intrauterine versus ectopic pregnancy.**

Key words*: Trophoblast, immunohistochemistry, ectopic pregnancy*

The usual histopathologic diagnosis of intrauterine pregnancy is made by demonstration of the chorionic villi. The chorionic villi may not be seen in the curettage material of intrauterine miscarriages in all cases. The cytokeratin (AE1/AE3) can stain three types of trophoblastic tissues. Immunohistochemical demonstration of the mononuclear intermediate trophoblastic cells may have diagnostic value when the chorionic villi are absent in the endometrial curretage material of the patients suspected of having miscarriage of an intrauterine pregnancy (1-4).

Ectopic pregnancy (EP) is a major cause of maternal morbidity and mortality. Although the treatment of this condition is primarily surgical, recently medical management has been favoured because of safety, effectiveness, cost-effectiveness and less morbidity. The patient will lose the chance of medical therapy and undergo surgery if the diagnosis of EP is delayed (1,2).

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Material and Method

The archival specimens of the patients were obtained from the Department of Pathology, Faculty of Medicine, Atatürk University, and 4 groups of patients were included in this retrospective study. Group1 (positive control) comprised of 10 patients who had miscarriage between the 5th and 12th weeks of gestation. These patients had chorionic villi in their endometrial curettage material. Included in group 2 (negative control) were the endometrial specimens of 5 non-pregnant patients who had endometrial curettage for menstrual irregularities. Group 3 (EP) comprised of 64 patients who had EP (mostly in the Fallopian tubes) between the 5th and 12th weeks of gestation. The patients underwent endometrial curettage and surgical termination of EP. The diagnosis of EP was confirmed by histopathologic and clinical means. There were 50 women in group 4 who were clinically suspected of having miscarriage while they had no chorionic villi, and cytio and syncytiotrophoblasts in their endometrial curettage material on routine H & E staining.

The uterine biopsy specimens were routinely processed and embedded in paraffin. All slides were examined for the followings: (1) Presence of intermediate trophoblastic cells by conventional light microscopy and immunohistochemistry. These cells have hyperchromatic nuclei with irregular outlines and frequent deep folds or clefts in the endometrium. It is difficult to differentiate them from the decidual cells by routine H & E staining. Cyto and syncytiotrophoblasts can be differentiated from the decidual cells on routine examination. Therefore, cytokeratin immunohistochemistry is needed to detect the intermediate trophoblastic cells. (2) Presence of hyaline within the spiral arterial walls; (3) Presence of linear and homogeneous eosinophilic deposits of fibrinoid material; and (4) The Arias-Stella phenomenon.

Table I. Histologic findings in curettage materials of intrauterine gestations without villi versus ectopic pregnancies.

	With villi (n=10) Group 1	Without villi (n=50) Group 4	(n=64) Group 3
Histologic features			
Intermediate trophoblast cells (immunohistochemically)	9	35	0
Hyaline spiral arteries	7	35	0
Fibrinoid	8	10	0
Arias Stella	10	12	42

After deparaffinization and rehydration, the histological sections were stained for cytokeratin (AE1-AE3) using the avidin-biotin technique. The slides were counterstained by Mayer's hematoxylin and cover-slides using Kaiser's gelatine.

Chi-Square test was used to compare the data of the groups.

Results

In group 1, 9 of 10 endometrial specimens obtained from miscarriages between 5 and 12 weeks of gestation showed strong staining for chorionic villi. These cells had a tendency to align around the endometrial vessels (Figure-1).

In group 2, the control endometrial specimens of the patients with menstrual irregularities were not stained by cytokeratin at all.

In group 3, the endometrial specimens of 64 cases with EP were not stained with cytokeratin stain either. Despite the presence of a prominent Arias Stella phenomenon in 42 of the patients, the glandular epithelium was almost free of non-specific staining.

In group 4, we identified intermediate trophoblast cells with cytokeratin immunohistochemistry in 35 of 50 endometrial curettage materials obtained from the intrauterine miscarriages without chorionic villi. In these 35 materials, the trophoblastic cells were found in the enlarged hyalinized vessels. In addition to that, 10 cases contained fibrinoid fragments with intermediate trophoblast cells. All specimens contained sufficient endometrial tissue for immunohistochemical investigation.

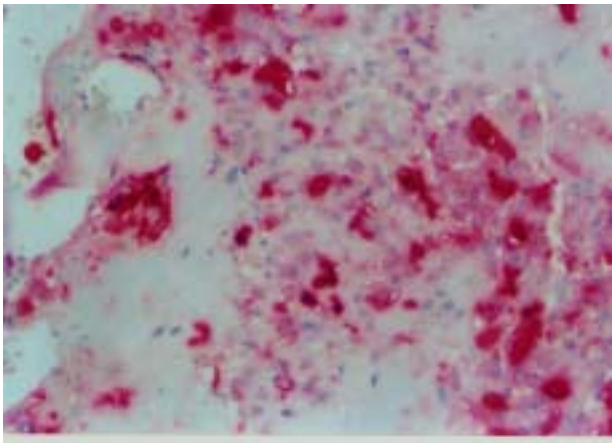

Figure 1. Keratin (AE1/AE3) immunoreactivity of intermediate trophoblastic cells invading the decidua (X200).

The salient microscopic features, which were significantly different between the groups are shown in Table I. For group 1 and 2 : Chi square= 10.5, p= 0,002, for group 3 and 4: Chi square= 94,67, p= 0,0001.

Discussion

The differential diagnosis between abortion and EP is of clinical importance (5-7). Recent studies suggest that chorionic villi may be absent in the endometrial curettage material in 31% of patients suspected of having uterine miscarriage (2,8). It was proposed that the presence of intermediate trophoblasts is the most useful histologic feature in confirming the presence of intrauterine gestation in the absence of chorionic villi in curettage material (9). Intermediate trophoblastic cells are difficult to distinguish from decidual cells by conventional light microscopy (2,9).

The immunohistochemical demonstration of cytokeratin is a useful tool for identifying patients who are suspected of having a miscarriage, particularly in the absence of chorionic villi in endometrial curretage materials (3,4). The use of human chorionic gonadotropin (hCG), human plasental lactogen (hPL), and pregnancy-specific beta-1- glycoprotein, as markers for trophoblastic tissue, is well documented in the literature (1-3). However, it is not widely recognized that cytokeratin is a very sensitive and reliable marker for all types trophoblastic tissues. Unlike hCG and hPL, which stain only the syncytiotrophoblast and intermediate trophoblast, but not cytotrophoblast; all three types of trophoblastic tissues can stain with cytokeratin staining (1,4).

Intact and fragmented epithelial cells lining the surface and glands of the endometrium usually stain positively with keratin, and should not be confused with intermediate trophoblastic cells invading the desidua and spiral

arterioles. Immunoperoxidase reactions for the localization of cytokeratin are useful because intermediate trophoblastic cells stain positively for cytokeratin, whereas decidual cells do not (2,9-11).

In this study, using cytokeratin markers, it was possible to detect the trophoblastic cells by hundred per cent in endometrial curettage materials of the patients with the clinical diagnosis of uterine miscarriage. Hyalinized vessels and fibrinoid, which are non-specific diagnostic features of intrauterine pregnancy, were also seen in the specimens (Table I) (2,12,13). Muijen et al. (14) showed that the placental villi in the endometrial curettage materials were cytokeratin positive.

Gruber et al. (13) proposed that when there is a high index of clinical suspicion, the finding of chorionic villi or an implantation site should not preclude further work up of a possible ectopic pregnancy. In cases where only a few villi or a single chorionic villus are identified, other signs of intrauterine implantation such as intermediate trophoblastic cells (using immunohistochemical cytokeratin), hyalinized vessels, and a fibrinoid matrix should be sought to establish firmly the diagnosis of an intrauterine pregnancy. However, according to our results, the patients who are suspected of having miscarriage of an intrauterine pregnancy while lacking villi in their endometrial curettage materials showed cytokeratin positivity. Our results proved the value of immunohistological techniques in the patients who were suspected of having miscarriage of an intrauterine pregnancy when there were no villi in their endometrial curettage materials.

In conclusion, a substantial proportion (%30) of patients with intrauterine miscarriage will not demonstrate chorionic villi in the initial histopathologic examination. The result of cytokeratin stain will help to determine whether the surgical intervention is necessary. The use of these hormone markers in endometrial specimens appears to increase our precision in the diagnosis of intrauterine or EP.

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