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Immunohistohemical evidences of pregnancy in uterine curettage tissue by the use of a double immunocytochemical staining technique using cytokeratin 7 and vimentin antibodies

Imunohistohemijsko dokazivanje trudnoće u kiretiranom tkivu uterusa pomoću tehnike dvostrukog imunocitohemijskog bojenja primenom antitetila na citokeratin 7 i vimentin

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Abstract

Background/Aim. Usual histopatological diagnosis of intrauterine pregnancy is made by demonstration of chorionic villi, but in the curettage tissue from intrauterine miscarriage they may not be present in all cases. The use of monoclonal antibody against cytokeratin as a sensitive and reliable marker for the morphologic discrimination between invasive trophoblastic (IT) cells and decidual cells has been well established. The aim of this study was to determine the presence of pregnancy in endometrial curettings when chorionic villi are absent from patients suspected of intrauterine pregnancy. **Methods.** Twenty cases of endometrial tissue specimens were investigated for cytokeratin and vimentin expression by a double immunostaining for detection of IT cells. **Results.** Out of the total number of cases (20) 17 cases expressed cytokeratin 7 positive IT cells, that are an evidence of pregnancy. **Conclusion.** The obtained results indicated, that double immunohistochemical demonstration of cytokeratin and vimentin is useful for identifying pregnancy in all chorionic villi-negative cases.

Key words:
abortion, spontaneous; immunohistochemistry; vimentin; keratins; trophoblasts.

Apstrakt

Uvod/Cilj. Patohistološka dijagnoza intrauterine trudnoće potvrđuje se prisustvom horionsnih čupica, ali one ne moraju biti prisutne u svim uzorcima kiretiranih tkivnih uzoraka endometrijuma. Korišćenjem monoklonskih antitela za citokeratin kao pouzdanih i osetljivih markera za morfološko razlikovanje invazivnih trofoblastnih ćelija i ćelija decidue u širokoj je upotrebi. Cilj ovog rada bio je da se utvrdi prisustvo trudnoće u slučajevima suspektne materične trudnoće, kada u kiretažnom materijalu nisu nađene placentne čupice. **Metode.** Kod 20 tkivnih uzoraka endometrijuma ispitivana je ekspresija citokeratina 7 i vimentina primenom dvostrukog imunološkog bojenja. **Rezultati.** Od ukupno 20 uzoraka 17 su pokazali ekspresiju citokeratin 7 pozitivnih ćelija invazivnog trofoblasta, što je znak evidentne trudnoće. **Zaključak.** Dvostruko imunohistohemijsko dokazivanje citokeratina 7 i vimentina može biti od značajne koristi u utvrđivanju prisustva trudnoće kada su placentne čupice otsutne.

Ključne reči:
abortus, spontani; imunohistohemija; vimentin; keratin; trofoblasti.

Introduction

The usual histopathologic diagnosis from intrauterine pregnancy is made by demonstration of the chorionic villi. Chorionic villi may not be seen in the curettage material of intrauterine miscarriage tissue in all cases. The presence of trophoblast in uterine curetting specimen is also an evidence of pregnancy. Although the morphology of trophoblast has been studied extensively and well described, recognition of invasive trophoblast (IT) cells intermingled with the decidual cells may be difficult, because a subset of the polyhedral IT cells is morphologically very similar to the decidual cells [1].

The use of monoclonal antibody against cytokeratin as a sensitive and reliable marker for the morphologic discrimination between IT cells and decidual cells has been well established [2].

For trophoblast, usually employed markers are the presence of cytokeratin 7, and the absence of vimentin. In contrast, for decidual cells are characteristic positive expression of vimentin and the absence of cytokeratin 7 [3].

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In this study we presented a double immunoenzymatic labelling to distinguish IT cells and decidual cells simultaneously in the same tissue sections.

Methods

The endometrial curettage material was obtained from 20 patient clinically suspected of having miscarriage, but with no chorionic villi in curettage tissue. We had two control groups. The positive control included of 10 patients with chorionic villi in their endometrial curettage material, and the negative control of 10 patients with uterine curettage for menstrual irregularities. The material was fixed in 10% buffered formalin, routinely processed, embedded in paraffin, cut and stained with haematoxylin-eosin (HE) and PAS.

Double immunostaining was performed as follows: the deparaffinized tissue sections were boiled in citrate buffer, pH 6.0, for 5 minutes, 3 times in microwave oven. The sections were first incubated with cytokeratin antibody (one part sections) and with vimentin antibody (second part sectons) in humidifed chamber at 4 °C overnight followed by PAP immunoperoxidase. The immunoreactivity was detected using 3-amino-9-ethylcarbazole (AEC) chromogen (red) [4].

After five washes in tris-buffered saline, the slides were incubated with vimentin antibody (one part sections) and with cytokeratin antibody (second-part sectons) at 37 °C for 60 minutes follwed by APAAP method [5]. The immunoreactive sites were detected with fast blue BB chromogen (blue). Finally, slides were mounted with an aqueous medium.

As control we used the alkaline phosphatase antialkaline phosphatase (APAAP) method. Primary monoclonal antibodies (cytokeratin 7 and vimentin) were incubated, after epitope retrieval with citrate buffer, in humidifed chamber at 4 °C overnight. Secondary and tertiary immunoreactions were performed at room temperature for 60 minutes. The antibody-antigen complexes were visualized by incubation for 20 minutes in new-fuchsin substrate (red). The sections were counterstained with haematoxylin and mounted in glycerol gelatine.

The antibodies and all other reagents were from DAKO, Glostrup, Denmark.

Results

Besides the characteristic growth pattern, IT cells are often difficult to recognize, because they closely resemble decidual cells on slides stained with HE or PAS (Figure 1).

The discrimination of decidual cells and IT cells is not difficult by the use of immunohistochemical staining of cytokeratin 7 and vimentin. The cytokeratin 7 immunoreactivity characterized IT cells as red intracytoplasmatic staining with new-fuchsin as chromogen (Figure 2). The IT cells as endovascular trophoblast were embeded in the wall of spiral artery as intramural trophoblast. The endovascular trophoblast intensively stained with anti-cytokeratin antibody were in contrast to the decidual cells which were cytokeratin negative (Figure 3). The immunostaining with antivimentin antibody as a marker for mesenchymal cells showed strong staining of decidual stromal cells, whereas glandular cells showed no vimentin expression (Figure 4).

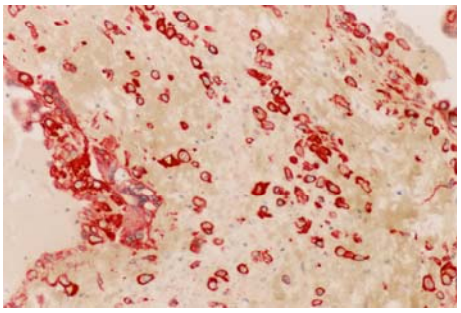

Fig. 1 – Invasive trophoblast cells are indistinct from decidual cells, a spiral artery in the center of the field (PAS; × 200)

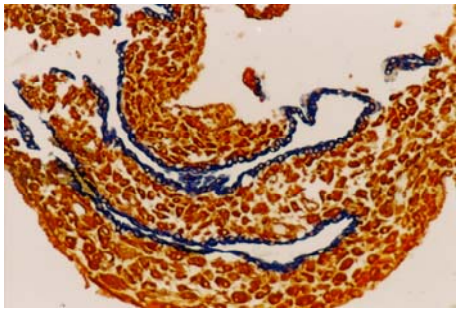

Fig. 2 – Strong cytokeratin 7 positive invasive trophoblast cells (APAAP; × 200)

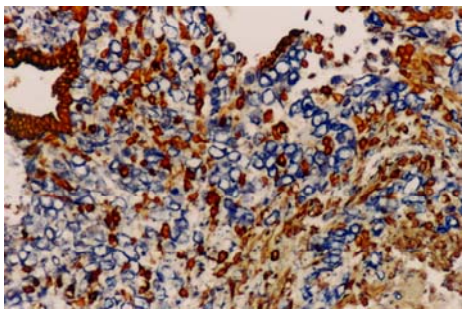

Fig. 3 – Endovascular intramural trophoblast, cytokeratin (APAAP; × 200)

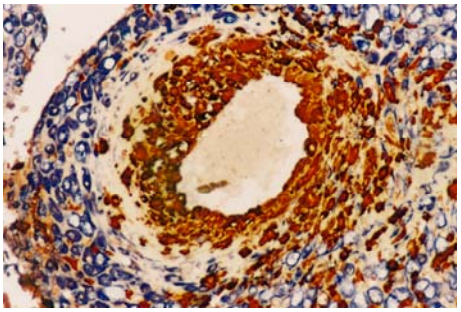

Fig. 4 – Strong vimentin positive decidual cells, glandular cells – negative (APAAP; × 200)

By the immunoenzymatic labelling two antigens (cytokeratin 7 and vimentin) localized in different cellular compartment (glandular and decidual cells) with two different

chromogen methods, we observed two separate colours (Figure 5). The IT cells occupying the cross sections of the vessel wall, demonstrated strong cytokeratin staining. The vimentin positive staining were present in decidual cells around spiral aretries (Figure 6).

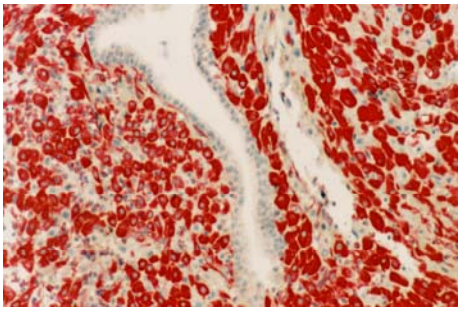

Fig. 5 – Double immunostaining: cytokeratin – endometrial glands (blue), vimentin – decidua (red) (PAP/APAAP; × 200)

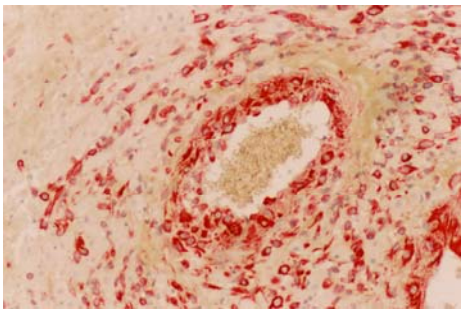

Fig. 6 – Double immunostaining: a spiral artery in the center of the field, invasive trophoblast cells cytokeratin-positive (red), decidual cells vimentin-positive (blue) (PAP/APAAP; × 200)

The cytokeratin immunostains both endometrial gland lining and scattered type of interstitial IT cells. The cytokeratin positive interstitial type of IT cells were dispersed among vimentin-positive decidual cells and formed a defined mosaic pattern (Figure 7).

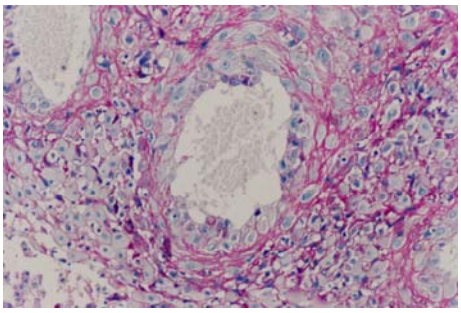

Fig. 7 – Double immunostaining: cytokeratin (red) positive interstitial type invasive trophoblast cells are dispersed among vimentin (blue) positive decidual cells (PAP/APAAP; × 200)

With double immunostain we identified IT cells in 17 out of 20 samples of endometrial curettage without chorionic villi.

Discussion

In the absence of chorionic villi, unequivocal trophoblastic cells are a convincing proof of pregnancy. Distinguishing trophoblast cells from decidual cells on morphologic grounds could be difficult.

Traditionally, two types of trophoblasts have been described: cytotrophoblast and syncytiotrophoblast.

Subsequent light microscopic, histochemical, electron-microscopic studies and immunocytochemical investigations have confirmed the presence of an invasive form of trophoblastic cells with characteristic morphologic and biochemical features. This third type of cells has been designated as invasive or intermediate trophoblast (IT) [6].

The first clear marker of an invasive trophoblast was described by Kurman et al [7], who demonstrated that first-trimester invasive trophoblasts react with anti-human placental lactogen antibodies. They coined the term "intermediate" invasive trophoblasts, partly because of their intermediate size between cyto- and syncytiotrophoblast.

Within implantation site of decidua several subsets of IT cells are present: interstitial trophoblast dispersed within decidua, and endovascular trophoblast which invades spiral aretries in the endometrium and myometrium, modifying them into noncontractile tubes allowing a stedy flow of maternal blood into the sinusoids [8]. The intravascular implantation site IT cells, formed cohesive cell aggregates in the wall and lumen of spiral aretries, demonstrated strong cytokeratin staining [9].

The utility of monoclonal antibody against cytokeratin for the morphologic discrimination between IT and decidual cells that is higher sensitivity compared to other immunostains has been well established. Anticytokeratin 7 antibody reacts with the 54 kDa cytokeratin intermediate filament protein, and it is shown in most glandular and ductal epithelia [10].

The decidua is a heterogeneous tissue which comprises not only the typical swollen stromal cells but also glands, blood vessels and numerous infiltrating cells. The decidual stromal cells are of mesenchymal origin. Antivimentin antibody reacts with the 57 kDa intermediate filament protein present in cells of mesenchymal origin [11].

The endometrial connective tissue was shown to contain vimentin intermediate filaments, whereas the invasive trophoblast showed no vimentin expression.

During the last decade the use of antibodies has been developed for both research and diagnostic purposes. However, in some cases there is a demand for detection of more than one antigen in a single tissue specimen. For a proper identification of co-localization and possible cell-to-cell spatial contacts, reliable double immunostaining is needed.

Conclusion

In this study, we demonstrated that cytokeratin and vimetin immunostains could be a standard for detection IT cells in the uterine curretage tissue.

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