IMMUNOPATHOLOGICAL STUDY OF HOFBAUER CELLS MORPHOLOGY AND DENSITY IN PLACENTAS FROM PREGNANCIES COMPLICATED WITH DIABETES MELLITUS


Introduction: Hofbauer cells have been described as frequent, pleomorphic cells of the villous stroma with round, fusiform, or stellate appearance. Their size depends on the length of their processes. The cells vary from 10 to 30 \( \mu \)m in diameter. Early studies had already found that the most striking aspects of Hofbauer cells are their highly vacuolated appearance and their granulated cytoplasm. Later investigations have pointed out that Hofbauer cells are characterized by numerous membrane-bound, electron-lucent vacuoles of different sizes, possessing amorphous material of varying density, dense granules (presumably lysosomes), and short profiles of endoplasmic reticulum.

Several theories have been reported trying to explain the origin of the Hofbauer cells. These include those proposed by Chaletzky who derived them from cells of maternal decidua; those by Neumann who considered them to be derivatives of the syncytiotrophoblast and an expression of malignancy; and other which supported that these cells could be derivatives of endothelial cells. A very important finding concerning the origin of Hofbauer cells was Wynn's observation, based on sex chromatin staining, that these are fetal cells. So, most investigators now believe that Hofbauer cells are of chorionic mesenchymal origin.

They can be recognized in placental villi at a very early stage of development. It has been supported that in placentas from uncomplicated pregnancies, Hofbauer cells either disappear or become scanty after the 4th to 5th month of gestation. On the other hand, in cases of pathological placentas due to intrauterine growth restriction or gestational diabetes mellitus their density seems to be increased. However, electron microscopy studies and immunohistochemistry revealed their presence throughout normal uncomplicated pregnancy as well, until term, and not only in immature villi of the center of the placentome.

Aim: The aim of this study was to investigate the differences in morphology and density of Hofbauer cells between placentas from normal term and pregnancies complicated with gestational diabetes mellitus. Additionally, the function of these cells, their macrophage character and their mitotic potential were examined.

Material and methods: This was a research immunopathological study, in which multiple sections of placental tissue were examined: i) 16 specimens were received from normal term pregnancies, while ii) 10 specimens were received from pregnancies between the 32nd and 38th week of gestation, associated with gestational diabetes mellitus.

Histological study of Hematoxylin - Eosin sections from formalin-fixed and paraffin-embedded placental tissues was performed for semi-quantitative determination of Hofbauer cells (H.c.) concentration per villus and their basic morphology. At least fifty villi per case were examined under high-power field (X400) observation. The density of Hofbauer cells per villus was determined by two independent observers and subsequently graded as 'focal' (+) (1-3 H.c./villus), 'intermediate' (++) (3-6 H.c./villus), or 'diffuse' (+++) (>6 H.c./villus).

Additional sections of each case were obtained for immunohistochemical investigation by Automated
Ventana Immunostainer. Slides were incubated with CD 68, A1 - Antichymotrypsine, Lysozyme, Ki-67, Cytokeratin - 7 and Vimentin.

Results: A focal presence of Hofbauer cells in mainly intermediate villi in 6/16 (37.5%) placental specimens of normal term gestations was observed during the basic H - E study. The rest 10 placental specimens were negative for Hofbauer cells presence at the basic study. However, additional immunohistochemical study revealed, via the macrophage marker A1 - Antichymotrypsine, focal density of Hofbauer cells in 14/16 (87.5%) specimens, while positive immunostain was noticed for the marker CD 68 as well in 50% of cases. Their morphological analysis showed cells with oviform shape, round nuclei and granulated cytoplasm. No mitotic activity was observed and the marker of cellular proliferation Ki-67 was positive in less than 5% of all examined specimens.

Both basic H - E study and immunohistochemistry, using the macrophage marker A1 - Antichymotrypsine, showed presence of Hofbauer cells in 7/10 (70%) placental specimens from pregnancies complicated with gestational diabetes mellitus. Immunohistochemistry failed to show H.c. presence in cases in which basic study was negative. In the vast majority of cases the density of H.c. was characterized as focal (6/7, 85.7%), while only in one case it was intermediate (1/6, 14.2%). Positive immunostain reaction was observed using the marker CD68 in 40% of cases. No positive reaction was noticed after the use of Vimentin, CK-7 or Ki-67 (<5%). The morphological characteristics of H.c. from pregnancies complicated with maternal diabetes mellitus did not present significant differences compared with those of same gestational age normal placentas.

Conclusions: Hofbauer cells are macrophages of fetal origin and can be found in human placental villi throughout pregnancy. The positive immunostain reaction of these cells after incubation with macrophage markers was characteristic, without significant differences between the two study groups. It seems that in cases of gestational diabetes mellitus, Hofbauer cells are more easily identifiable via basic H - E study because of the oedematous morphology of the villi which can unmask numerous macrophages. In both groups, A1 - Antichymotrypsine was recognized as the most specific marker for Hobauer cells determination, as it revealed their presence even in cases in which basic H - E study was negative.