



## The Number of HOFBAUER Cells Increases in Preeclamptic Placenta:an Immunohistochemical Study

## KEYWORDS

Placenta,Preeclampsia,Hofbauer cells,CD 68 macrophage marker

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**ABSTRACT** *OBJECTIVE:*Hofbauer cells are fetal macrophages residing in the stroma of the chorionic villi of the human placenta.These cells are thought to function in many processes, but the possible role of hofbauer cells in placenta is unclear.Hofbauer cells are located close to trophoblast cells and fetal capillaries, changes in distribution or phenotype of these cells may be involved in the pathogenesis of preeclampsia. The aim of this study was to quantify the number of hofbauer cells in preeclamptic placental tissue compared with normal. *MATERIALS AND METHODS:*Placentas of control(n=50) and preeclampsia(n=50) pregnancies were sequentially immunohistochemically stained for CD 68( macrophage marker). Results were evaluated in a semi-quantitative method. *RESULTS:*CD68 cells increased significantly in preeclamptic placentas compared with control( $6.9 \pm 1.07$  and  $3.8 \pm 0.91$  respectively) . *CONCLUSION:*We hypothesized that the increased number of hofbauer cells in preeclamptic placenta may be involved in the pathogenesis of preeclampsia.

**INTRODUCTION:**

Preeclampsia is a pregnancy specific disorder with clinical manifestation of new onset of maternal hypertension,proteinuria and edema.Preeclampsia affects 5% to7% of all pregnancies that results in morbidity of both mother and fetus<sup>1,2</sup>. The only treatment for this disease is delivery of placenta, implicates the placenta as a critical factor for the pathogenesis of the disease.

The placenta has two major cells ,trophoblast and hofbauer cells.The trophoblast are the outer covering cells of the chorionic villi.There are numerous studies regarding trophoblast cells ,only few studies on hofbauer cells.Hofbauer cells are fetal macrophages in the stroma of the chorionic villi .These cells are located close to trophoblast cells and fetal capillaries.Hofbauer cells express and produce VEGF(vascular endothelial growth factor) and involves in the process of vasculogenesis and angiogenesis<sup>3</sup>.In the first trimester placenta hofbauer cells in the placental villous core are in close vicinity to angiogenic cell cords and primitive vascular tubes with the number of hofbauer cells being significantly correlated with vasculogenic structures<sup>4</sup>.In the present study we observed an alteration in the number of hofbauer cells which may be involved in the pathogenesis of preeclampsia.

**MATERIALS AND METHODS:**

A total of 100 placental tissues from uncomplicated and preeclampsia pregnancies were included in the study. These were collected from the SRM University, hospital, department of gynecology and obstetrics. Informed consent forms and protocols were approved by the institutional ethical committee. Placental tissues were divided in two study groups. The control/normotensive group consisted of placental tissues collected from 50 women with uncomplicated pregnancies who had normal blood pressure and no proteinuria. The preeclampsia group contained tissues collected from 50 women in whom preeclampsia was defined as a blood pressure of >140/90mm Hg, and with proteinuria >300mg/l in a 24hr urine collection. Samples (1.5x1.5x1cm in diameter) taken from the maternal surface of each placenta; infarct areas were excluded from the study.

**Immunohistochemistry:**

Expression of CD68 was analyzed in 100 placental tissues. All tissues were fixed in formalin, embedded in paraffin, and cut into 5µm thick sections, which were collected on slides coated with poly-L-lysine. After the paraffin was removed, the sections were rehydrated.Immunostaining was performed by the streptavidin-biotin-peroxidase method. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide.Antigen retrieval was carried out in a microwave oven for 15 minutes in 10 nM citrate buffer(pH 6.0) for CD 68. Sections were incubated overnight at 4°C with mouse monoclonal anti-human CD 68 (M0814;clone KP,dilution 1/200;dako,glostrup,Denmark) for identification of placental macrophages.Finally slides were incubated with the biotinylated secondary antibody (TP-060-HL;lab vision,Fremont,CA,USA) and a streptavidin-peroxidase complex(TP-060-HL;Lab vision),respectively, for 15min.The resulting signal was developed with diaminobenzidine(DAB) .Each step was followed by three washes in phosphate buffered saline(PBS).Sections were counterstained with hematoxylin and mounted.

**Evaluation of immunohistochemical staining:**

The number of hofbauer cells were counted randomly in 5 fields of each sections by two investigators blinded to the study in the CD 68 stained placental tissue sections.

**Statistical analysis:**

Statistical analysis was carried out using SPSS for windows(version 13.0,Chicago IL,USA).Data were expressed as mean ± standard deviations. The differences of groups were analyzed by Mann-whitney U test.P value < 0.05 was considered statistically significant.

**RESULTS:****COMPARISON OF NUMBER OF CD68 CELLS BETWEEN PREECLAMPSIA AND CONTROL:**

The number of hofbauer cells increased significantly in preeclampsia compared with control placentas ( $6.9 \pm 1.07$  and  $3.8 \pm 0.91$  respectively).Fig (A & B)

**DISCUSSION:**

Preeclampsia is a systemic disorder which originates in the

placenta, starting with inadequate cytotrophoblast invasion and ending with maternal endothelial dysfunction. Recent studies have shown that excessive maternal systemic inflammatory response to pregnancy and uteroplacental hypoxia might play a major role in the disease<sup>5</sup>.

In the present study we found increased number of hofbauer cells in preeclamptic placenta compared to control.

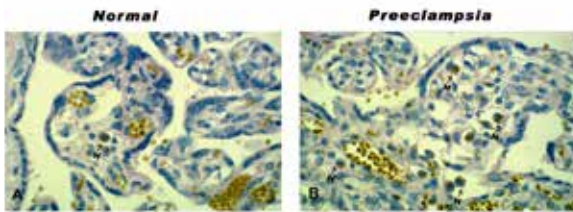
The hofbauer cells are resident fetal tissue macrophages in the stroma of the chorionic villi of the human placenta. Their population remains constant throughout gestation, occupying around 40% of villous stromal cells<sup>6</sup>. Evsen MS et al showed increased number of hofbauer cells in severe preeclampsia with HELLP syndrome. These increased hofbauer cells may be associated with increased inflammation or may have an adaptive mechanism at the fetal site of the placenta in patients with HELLP syndrome<sup>7</sup>. Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and thromboxane (TXA<sub>2</sub>) were produced by hofbauer cells with PGE<sub>2</sub> synthesis being predominant. Culturing hofbauer cells in low oxygen showed a decreased production of PGE<sub>2</sub> with TXA<sub>2</sub> synthesis remaining unchanged<sup>8</sup>.

Therefore we hypothesized that the increased number of hofbauer cells in preeclamptic placenta may be involved in the pathogenesis of the disease.

#### LEGENDS:

**Fig 1: Increased number of hofbauer cells (CD68 cells) in preeclamptic placentas. A. Normal B. Preeclampsia. 40x.**

**Fig 1: Increased Number of hofbauer cells (H) in Preeclamptic placentas Compared with normal**



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