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HISTOCHEMICAL STUDY ON THE EXPRESSION OF SFLT-1 IN HOFBAUER CELLS PRESENT IN THE PLACENTA OF PREECLAMPTIC PREGNANCIES

Anatomy

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ABSTRACT

Introduction: Soluble fms-like tyrosine kinase-1 (sFLT-1) is an antiangiogenic protein implicated in the pathogenesis of preeclampsia. In preeclampsia, the placenta produces excessive sFLT-1, resulting in endothelial dysfunction. However, its expression in Hofbauer cells of preeclamptic placentas has not been extensively studied. **Aim:** The present study aimed to correlate the number of Hofbauer cells present in placentas from preeclamptic pregnancies with the level of sFLT-1 expression and compare them with normal placentas. **Materials and Methods:** A total of 100 placentas were collected, including 50 normal placentas and 50 placentas from preeclamptic pregnancies. Tissue sections were subjected to immunohistochemical staining using CD68 to identify Hofbauer cells and sFLT-1 antibodies to assess protein expression. Staining intensity was scored semiquantitatively (0-3) by two pathologists blinded to the study groups. **Results:** The number of Hofbauer cells was significantly increased in preeclamptic placentas compared to normal placentas (6.9 ± 1.07 vs. 3.8 ± 0.91). Similarly, sFLT-1 expression in Hofbauer cells was significantly higher in the preeclampsia group than in controls (250 ± 18.38 vs. 153 ± 37.78). **Conclusion:** The present study demonstrated a significant increase in both the number of Hofbauer cells and the expression of sFLT-1 in preeclamptic placentas. These findings suggest that Hofbauer cells may serve as an additional placental source of sFLT-1 secretion in preeclampsia.

KEYWORDS

Placenta; Preeclampsia; Soluble Fms-Like Tyrosine Kinase-1 (sFLT-1); Hofbauer Cells; CD68.

INTRODUCTION

Hofbauer cells are fetal placental macrophages located within the stroma of chorionic villi. These cells originate from mesenchymal precursor cells during early pregnancy and are situated close to trophoblasts and fetal blood vessels[1]. Hofbauer cells appear in chorionic villi approximately 18 days after conception[2].

In normal pregnancy, Hofbauer cells are predominantly M2-polarized macrophages characterized by spindle-shaped morphology and cytoplasmic vacuoles. These cells play an important role in maintaining maternal-fetal immune tolerance and contribute to trophoblast function, tissue remodeling, vasculogenesis, and angiogenesis[3,4].

Hofbauer cells are closely associated with angiogenic cell cords and primitive vascular structures in placental villi. They secrete vascular endothelial growth factor (VEGF), VEGF receptors, and fibroblast growth factor-2 (FGF-2), thereby promoting placental vascular development [5,6].

Placental development involves both vasculogenesis and angiogenesis. Vasculogenesis refers to the formation of blood vessels from precursor cells, whereas angiogenesis involves the growth of new vessels from pre-existing vasculature. These processes are regulated by VEGF and its receptors, Flt-1 and Flk-1[7,8]. During early placental development, VEGF is primarily secreted by cytotrophoblasts; however, as gestation progresses, Hofbauer cells become an important source of VEGF production.

Preeclampsia is a multisystem disorder characterized by hypertension and proteinuria after 20 weeks of gestation. It affects approximately 5%-7% of pregnancies worldwide and is associated with significant maternal and fetal morbidity and mortality[9-11]. The disorder is believed to arise from abnormal trophoblastic invasion of spiral arteries, leading to placental hypoxia and endothelial dysfunction[12-16].

Soluble fms-like tyrosine kinase-1 (sFLT-1) is a soluble VEGF receptor that lacks transmembrane and cytoplasmic domains but retains the extracellular ligand-binding domain. sFLT-1 acts as an antiangiogenic factor by binding VEGF and placental growth factor (PLGF), preventing their interaction with proangiogenic receptors[17,18]. Elevated circulating levels of sFLT-1 have been reported in women with preeclampsia, and experimental administration of sFLT-1 in pregnant animal models induces hypertension and proteinuria[19-23]. Previous studies have identified syncytiotrophoblasts and syncytial knots as major sources of placental sFLT-1 production[24]. The present study was designed to investigate whether Hofbauer cells also express sFLT-1 and contribute to its increased levels in preeclampsia.

MATERIALS AND METHODS

Study Design and Sample Collection

This study was conducted in the Department of Anatomy at SRM Medical College Hospital and Research Centre, Potheri, India. A total of 100 placentas were collected immediately after delivery and fixed in 10% formalin.

The samples were divided into two groups:

- Control group: 50 placentas from normotensive women with uncomplicated pregnancies
- Preeclampsia group: 50 placentas from women diagnosed with preeclampsia

Preeclampsia was defined as blood pressure >140/90 mmHg associated with proteinuria >300 mg/24 hours.

Patients with chronic hypertension, renal disease, diabetes mellitus, or pre-existing proteinuria were excluded.

Ethical approval was obtained from the Institutional Ethics Committee of SRM University, and informed consent was obtained from all participants.

Placental tissue samples measuring approximately 1.5 × 1.5 × 1 cm were collected from the maternal surface while avoiding infarcted areas.

Immunohistochemistry

CD68 Immunostaining

Serial sections of 5 µm thickness were mounted on poly-L-lysine-coated slides. Sections were deparaffinized, rehydrated, and subjected to antigen retrieval using citrate buffer (pH 6.0) in a microwave oven. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide.

Sections were incubated overnight at 4°C with mouse monoclonal anti-human CD68 antibody (clone KP1; Dako, Denmark; dilution 1:200). Detection was performed using biotinylated secondary antibody and streptavidin-peroxidase complex. Diaminobenzidine (DAB) was used as the chromogen, followed by hematoxylin counterstaining.

sFLT-1 Immunostaining

Paraffin sections were deparaffinized and rehydrated. Antigen retrieval was performed using sodium citrate solution. Endogenous peroxidase activity was quenched using hydrogen peroxide.

After blocking with normal goat serum, sections were incubated overnight with primary antibody against soluble VEGFR-1 (sFLT-1; dilution 1:150). Slides were then incubated with goat anti-rabbit secondary antibody and avidin-biotin complex. DAB chromogen was used for visualization, followed by hematoxylin counterstaining.

Evaluation of Immunostaining

The intensity of sFLT-1 staining in Hofbauer cells was independently evaluated by two investigators blinded to the study groups.

Immunoreactivity was graded semiquantitatively as follows:
- 0 = Negative
- 1+ = Weak
- 2+ = Moderate
- 3+ = Strong

The number of CD68-positive Hofbauer cells was counted in five randomly selected microscopic fields.

An H-score was calculated using the formula:
H-score=$\sum$(I×PC)

where I represent staining intensity and PC represents the percentage of positively stained cells.

Statistical Analysis
Data were analyzed using SPSS version 20.0 (IBM Corp., Chicago, IL, USA). Results were expressed as mean ± standard deviation. Statistical comparisons between groups were performed using the Mann-Whitney U test. A p-value < 0.05 was considered statistically significant.

RESULTS
Patients in the preeclampsia group demonstrated significantly lower gestational age, APGAR score, placental weight, and birth weight, along with higher maternal age compared to the control group.

Table 1. Patient Characteristics

Parameter	Control (Mean ± SD)	Preeclampsia (Mean ± SD)
Maternal age	38 ± 1.55	40 ± 1.90
Gestational age	39 ± 1.55	35 ± 2.3
APGAR score (1 min)	7.8 ± 0.3	6.3 ± 1.7
APGAR score (5 min)	8.8 ± 0.3	7.2 ± 1.9
Placental weight	449 ± 48.65	308 ± 68.15
Birth weight	3024 ± 234	1986 ± 271

The number of CD68-positive Hofbauer cells in chorionic villi was significantly higher in preeclamptic placentas compared to controls (6.9 ± 1.07 vs. 3.8 ± 0.91) Fig 1. (A&B) (Table2). Similarly, sFLT-1 expression in Hofbauer cells was significantly increased in the preeclampsia group (250 ± 18.38 vs. 153 ± 37.78) Fig 1. (C&D) (Graph1).

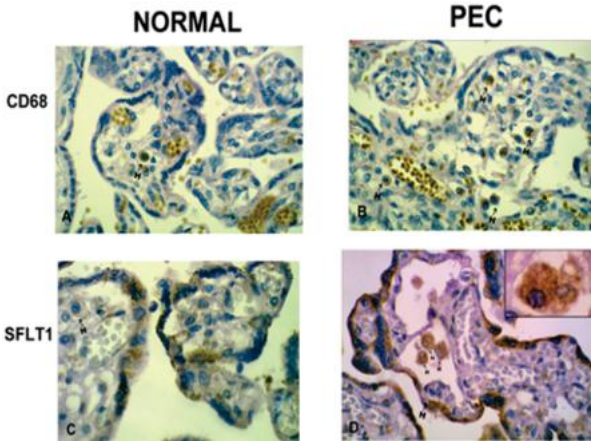
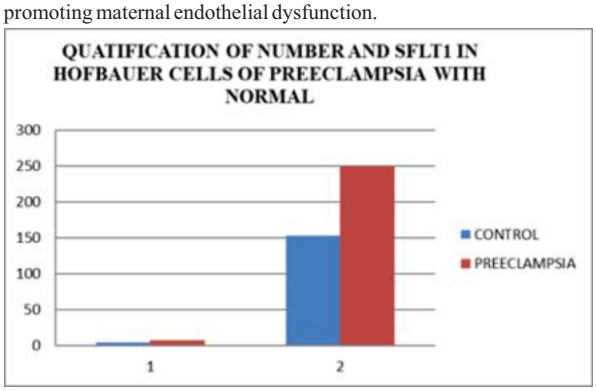


Figure 1:
(A) Decreased number of CD68-positive Hofbauer cells in normal placenta.
(B) Increased number of Hofbauer cells in preeclamptic placenta.
(C) Reduced expression of sFLT-1 in Hofbauer cells of normal placenta.
(D) Increased expression of sFLT-1 in Hofbauer cells of preeclamptic placenta.

Table 2. Immunohistochemical Findings

Parameter	Control	Preeclampsia
CD68-positive Hofbauer cells	3.8 ± 0.91	6.9 ± 1.07
sFLT-1 expression	153 ± 37.78	250 ± 18.38

Placental hypoxia in preeclampsia may contribute to increased Hofbauer cell proliferation and enhanced sFLT-1 expression, thereby promoting maternal endothelial dysfunction.



Graph:1

DISCUSSION
Hofbauer cells are specialized fetal macrophages located within the stromal core of placental chorionic villi and play a crucial role in placental development through their involvement in vasculogenesis, angiogenesis, immune regulation, and tissue remodeling. Under physiological conditions, these cells contribute to the maintenance of fetal-maternal immune tolerance by secreting anti-inflammatory cytokines and growth factors that support normal placental vascular development. However, alterations in their number and functional phenotype have been implicated in several placental pathologies, including preeclampsia[25].

The present study demonstrated a significant increase in the number of Hofbauer cells in preeclamptic placentas compared with normal placentas. In addition, immunohistochemical analysis revealed significantly enhanced expression of soluble fms-like tyrosine kinase-1 (sFLT-1) within these cells. These findings suggest that Hofbauer cells may actively participate in the pathogenesis of preeclampsia by contributing to the excessive production of anti-angiogenic factors.
Preeclampsia is characterized by an imbalance between pro-angiogenic and anti-angiogenic factors, with elevated circulating sFLT-1 being one of the major contributors to endothelial dysfunction. sFLT-1 is a soluble splice variant of vascular endothelial growth factor receptor-1 (VEGFR-1) that binds vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), thereby preventing their interaction with endothelial receptors. The resulting reduction in angiogenic signaling contributes to impaired placental vascular development, maternal endothelial dysfunction, hypertension, and proteinuria, which are the hallmark features of preeclampsia[26].

Rajakumar et al. reported that syncytiotrophoblasts are the principal source of placental sFLT-1 and demonstrated strong sFLT-1 expression in these cells. They further proposed that syncytiotrophoblast-derived microparticles and syncytial fragments released into the maternal circulation contribute substantially to the elevated circulating concentrations of sFLT-1 observed in women with preeclampsia. While trophoblasts have long been considered the primary source of placental sFLT-1, accumulating evidence indicates that other placental cell populations may also contribute to its production[27].

Several studies have shown that cells of the monocyte/macrophage lineage, including peripheral blood mononuclear cells (PBMCs), circulating monocytes, and tissue macrophages, express FLT-1 and are capable of producing sFLT-1[28]. Ahmed et al. demonstrated immunoreactivity for FLT-1 in Hofbauer cells within chorionic villi, suggesting that fetal macrophages possess the molecular machinery necessary for sFLT-1 synthesis[29]. Likewise, Di Marco et al. reported increased sFLT-1 expression in circulating monocytes of patients with chronic kidney disease, indicating that activated monocytes/macrophages can serve as an important source of this anti-angiogenic protein in inflammatory conditions[30].

The findings of the present study are consistent with these previous reports and provide further evidence supporting the role of Hofbauer cells in the pathophysiology of preeclampsia. The observed increase in both Hofbauer cell number and sFLT-1 immunoreactivity suggests that these fetal macrophages may undergo activation or proliferation in response to the hypoxic and inflammatory microenvironment

characteristic of preeclamptic placentas. Placental hypoxia, oxidative stress, and increased levels of inflammatory cytokines may stimulate Hofbauer cells to produce greater amounts of sFLT-1, thereby amplifying the anti-angiogenic state and further impairing placental vascular function. This mechanism may establish a vicious cycle in which placental ischemia promotes macrophage activation, increased sFLT-1 production, and worsening endothelial dysfunction.

Collectively, these findings support the hypothesis that Hofbauer cells represent an important additional source of placental sFLT-1 beyond syncytiotrophoblasts. Their increased number and enhanced expression of sFLT-1 in preeclamptic placentas suggest that fetal macrophages may play a significant role in the development and progression of the disease. Further molecular studies evaluating macrophage activation markers, cytokine profiles, and gene expression are warranted to clarify the precise mechanisms regulating sFLT-1 production by Hofbauer cells and to determine whether modulation of Hofbauer cell activity could serve as a potential therapeutic strategy for preeclampsia.

CONCLUSIONS

The present study demonstrated a significant increase in both the number of Hofbauer cells and the expression of sFLT-1 in preeclamptic placentas. These findings suggest that Hofbauer cells may represent an additional placental source of sFLT-1 secretion in preeclampsia and may contribute to disease pathogenesis.

Conflicts of Interest: Nil

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