Middle East Journal of Science

https://dergipark.org.tr/mejs

MEJS

e-ISSN:2618-6136

Research Article

THE CHANGES IN HOFFBAUER AND SYNCYTIOTROPHOBLAST CELLS IN SERIOUS PREECLAMPSIA COMPLICATED WITH HELLP SYNDROME (ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL STUDY)

***Yusuf NERGİZ*[1*] *Şebnem NERGİZ*[2] *Fırat AŞIR*[1] *Engin DEVECİ*[1] *Erdal SAK*[3] *Sıddık EVSEN*[3] *Selçuk TUNİK*[1] *Uğur ŞEKER*[1]**

[1]Department of Histology and Embryology, Faculty of Medicine, Dicle University, Diyarbakır, Turkey
[2]Department of Microbiology, Atatürk Health High School, Dicle University, Diyarbakır, Turkey
[3]Department of Obstetrics and Gynecology, Faculty of Medicine, Dicle University, Diyarbakır, Turkey
*Corresponding Author: email: yusufnergiz21@gmail.com

Abstract: *HELLP syndrome is a syndrome characterized by hemolytic anemia, increased liver enzymes, and thrombopenia and can be seen in 1% of all pregnancies, 10-20% of pregnancies with pain, preeclampsia, and eclampsia. HELLP syndrome usually develops in the third trimester and its pathogenesis is not clear. Human placental villus stroma contains macrophages called Hoffbauer cells (HC), which are thought to be involved in many processes. HC is also called placental macrophage and has a role in many placental events. This study aimed to evaluate the immunohistochemistry and ultrastructural of syncytiotrophoblast and Hoffbauer cells in the placental villi of HELLP syndrome patients. In our study, placental tissues obtained from human normal and HELLP syndrome pregnancies were prepared for light and transmission electron microscopy (TEM) studies. Immunohistochemistry techniques were applied to placenta sections. HC localizations were determined with CD68 (Hoffbauer cell marker). Fine structure properties of HC and syncytiotrophoblasts were examined by TEM. When the HELLP group fetal placental sections were examined under the light microscope, intracytoplasmic edema in syncytiotrophoblast, degenerative vacuoles, and degenerative findings on cell surface membranes were observed. Moreover, villous edema was remarkable. The increase in the number of Hoffbauer cells per villus in the HELLP group was statistically significant ($p<0.00$). Compared with the control group, there was a significant increase in the number of Hoffbauer cells and syncytiotrophoblast in HELLP group, and also degenerative changes were observed in the ultrastructural structure of these cells.*

Keywords: *HELLP, Hoffbauer cell, syncytiotrophoblast, immunohistochemistry, placenta.*

Received: August 25, 2022 *Accepted: November 22, 2022*

1. Introduction

Since the middle of the 19th century, there have been many studies on the presence of large cells in the stroma of human placental chorionic villi. It was Kastschenko who pointed out the presence of these cells in the villous stroma. After 1885, researchers named Virchow, Chaletzky, and Neumann discovered large isolated cells with clear cytoplasm in hydatiform molar pregnancies. For this reason, these cells were called Chaletzky-Neumann cells in the past. Later, at the beginning of the 20th century, it was the researcher named Hoffbauer who best described the functional and morphological features of these cells in normal chorionic villi. For this reason, the term Hoffbauer cell has been widely used in the literature [1].

Hoffbauer cells in the villous stroma are round, pleomorphic, or star-shaped. They are 10-30 μm in diameter and are elongated cells. The prominent feature of these cells in early studies is that they have a vacuolated and granular cytoplasm [2]. Later, researchers reported that Hoffbauer cells have multiple membranes, electron-lucent vacuoles of varying size, dense granules with amorphous material, and short endoplasmic reticulum [3-4].

HELLP syndrome is generally considered to be a variant or complication of preeclampsia. It is a life-threatening obstetric complication [1]. The placenta plays a vital role in the nutritional transport between mother and fetus during pregnancy. The main cell type in the placenta is syncytiotrophoblast cells which are located in the intervillous space and in contact with maternal blood. Besides these cells, numerous fibroblast cells adjacent to the fetal capillaries, Hoffbauer cells, and tissue macrophages are also present [4]. There are two major cells in the placenta, trophoblast and Hoffbauer cells. Although there are many studies on trophoblasts surrounding chorionic villi, few studies are about Hoffbauer cells. Hoffbauer cells are fetal tissue macrophages in the chorionic villous stroma. These cells are located close to the trophoblast and fetal capillaries.

Hoffbauer cells express vascular endothelial growth factor [VEGF] that plays a role in the development of vasculogenesis and angiogenesis [5]. Hoffbauer cells in placental villi are located in the immediate vicinity of angiogenic cell cords and primitive vascular tubes [6]. Hoffbauer cells are placental macrophages present in the villi of the placenta during pregnancy. These cells are normally generated on the 18th day of pregnancy and function in the placenta until the end of pregnancy. The cellular origin of the Hoffbauer cells in the placenta varies during pregnancy. In the early stages of pregnancy, they originate from villi mesenchymal cells, but through the end of pregnancy derived by the transformation of fetal monocytes [7]. The role of Hoffbauer cells in the placenta has not been fully elucidated. However, an increase in Hoffbauer cells has been reported in various placental inflammation cases, particularly villitis. Syncytiotrophoblasts are formed by cellular fusion rather than by cellular division. They are continuous, acellular system and their boundaries are not clear [8]. The surface of these cells has irregular microvilli. The luminal cytoplasm contains vesicles surrounded by flat membranes. The remaining cytoplasm contains a large number of rough and smooth endoplasmic reticulum, a well-developed Golgi complex, and numerous mitochondria.

In this study, we aimed to investigate the immunohistochemistry and ultrastructural of the syncytiotrophoblast and Hoffbauer cells in the placental villi of pregnancies with HELLP syndrome.

2. Materials and Methods

The study was approved by the Dicle University Faculty of Medicine non-invasive clinical research ethics committee (record number: 456, date: 28.03.2012). This study was funded by the Dicle University Scientific Research Platform with grant number:13-TF-89. Twenty-five patients with HELLP syndrome and twenty-five healthy pregnant women, a total of 50 pregnant women, were included. Placental samples were obtained from the department of Obstetrics and Gynecology, Faculty of Medicine, Dicle University from women at 36 to 39 weeks of pregnancy. Criteria for HELLP syndrome were assigned as systolic/diastolic blood pressure (BP): 140/90 mmHg and proteinuria>300 mg in 24h.

2.1. Histologic technique

Placental tissue samples were fixed in 10% neutral formalin solution and a paraffin-embedding wax protocol was performed. 5 μm sections were taken for histological and for immunohistochemical staining. Sections were immunostained with Hoffbauer cell marker CD68 and two separate blinded researchers counted placental villi and Hoffbauer cells in the same areas. The mean number of Hoffbauer

cells per villi was determined. The data were analyzed by Student's t-test by the SPSS program, and the number of Hoffbauer cells was calculated in each group. Sections were evaluated by Zeiss imager A2 light microscope, and photomicrographs were taken.

2.2. Immunohistochemical technique

Placental samples were fixed in a 10% neutral formalin solution and processed for routine histological tissue processing. Sections were deparaffinized in xylene and brought to distilled water. To remove epitope blocking, samples were boiled in citrate buffer solution (pH:6.0) in a microwave oven at 700 W. 0.1% hydrogen peroxide was used to block endogenous peroxidase activity for 20 minutes. The blocking solution (Cat.No:85-9043, Invitrogen, USA) was done for 10 minutes. Sections were incubated at 4°C overnight with anti-CD68 (catalog no: ab125212, Abcam, 1:750). A secondary antibody (Invitrogen, USA) was applied for 20 minutes. Slides were then exposed to streptavidin peroxidase for 20 minutes. Diaminobenzidine (Invitrogen, USA) was used as chromogen. Sections were counterstained with hematoxylin and mounted with a mounting medium.

2.3. TEM technique

In order for the Transmission of an Electron Microscope (TEM), placental tissue samples were fixed in 2.5% buffered glutaraldehyde and then, in 1% osmium tetroxide for routine electron microscopic procedure. Semi-thin sections cut with Leica ultra-cut R ultramicrotome were stained with toluidine blue. Semi-thin sections on copper grids were counterstained by uranyl acetate-lead citrate. The grids were evaluated in Jeol 1010 transmission electron microscope, and micrographs were taken.

2.4. Statistical analysis

Statistical analysis was performed by using IBM SPSS version 25 software. Shapiro Wilk test was used for data contribution. Student's test–test and Mann-Whitney U test were used to compare the binary group averages. $P<0.05$ was considered statistically significant.

3. Results

3.1. Immunohistochemical findings

In the sections stained with CD68 antibody, the positive Hoffbauer cell number per villus was 0.23 ± 0.02 in the control group (Fig.1A) and 0.83 ± 0.12 in the HELLP group (Fig.1B). The number of Hoffbauer cell counts per villi in HELLP group placentas (Fig.2) was statistically significant ($p < 0.001$).

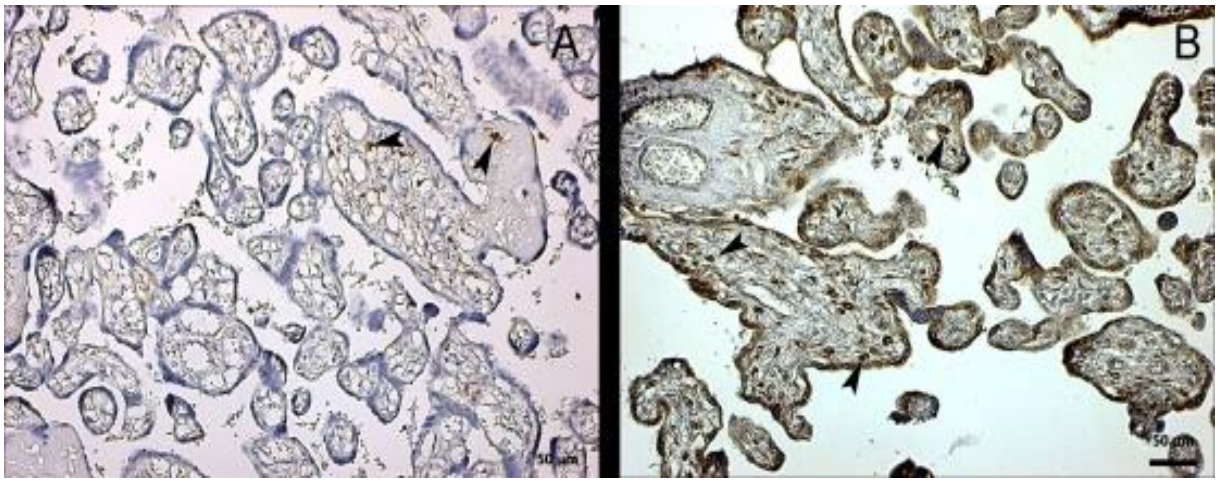

Figure 1. Compared to the control group, a significant increase in the number of CD 68 positive Hoffbauer cells per villus was observed in the placental villi of HELLP group. **A)** Control group, **B)** HELLP group (Staining: IHC CD 68, counterstaining with Hematoxylin, Bar: 50 µm).

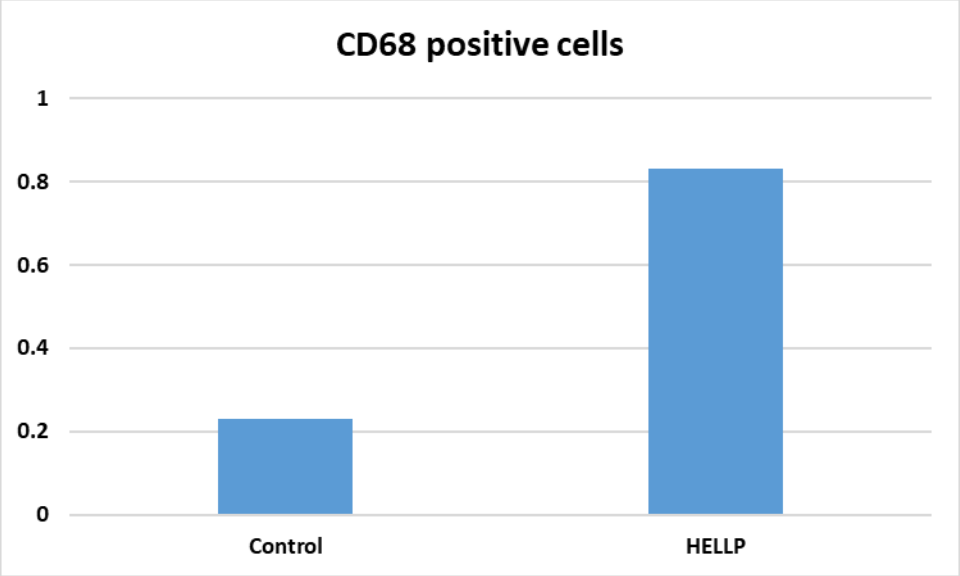

Figure 2. Graph showing the percentage of Hoffbauer cells per villus in control and HELLP group placentas.

3.2. Ultrastructural Findings

Syncytial nodes, syncytiotrophoblasts, Hoffbauer cells, and capillary endothelial structures were observed to have a normal histological structure in the control group of placental sections. Ultrastructural findings of cells in the placental villi of HELLP group. In the placental sections of the HELLP group, intracytoplasmic edema, degenerative vacuoles, and degenerative findings in cell surface membranes were observed in syncytiotrophoblasts. In addition, villous edema was prominent. In another placenta section of the same group, as well as intravascular coagulation, the presence of red blood cells in the extravascular areas due to endothelial degeneration, thinning of the capillary endothelium, villous edema, and degenerative vacuoles were observed (Figure 3).

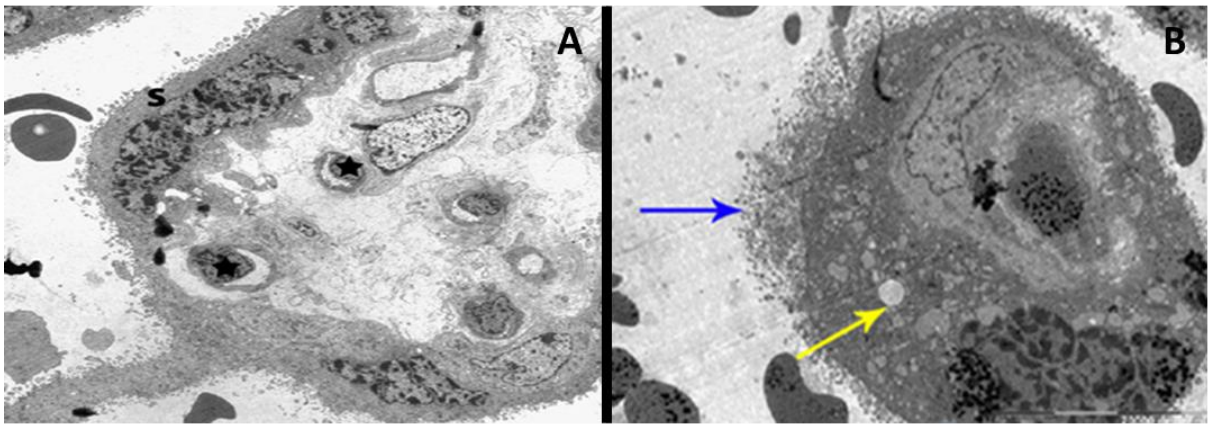

Figure 3. A) In the control group placental section, a structural histologic view to intensive microvilli on the surface of syncytiotrophoblasts, (S), and capillary endothelium cells (star) are seen. **B)** HELLP group placenta section. Degenerative structure (blue arrow) in cell surface membrane, intracytoplasmic edema, and degenerative vacuoles (yellow arrow) in syncytiotrophoblast (uranyl lead nitrate-uranyl acetate, Bar: 20000 nm)

4. Discussion

Preeclampsia is a clinical complication that starts in the 20th week of the pregnancy and continues until 4–6 weeks after birth. Preeclampsia is characterized by hypertension and proteinuria [11]. There are many risk factors that lead to preeclampsia such as abnormal trophoblastic invasion, endothelial dysfunctişon, impaired nitric oxide and lipid metabolism, and genetic and nutritional factors [12].

Immune tolerance at the feto-maternal junction of the placenta is a complex phenomenon. Although much is known about immune-capable macrophages in the maternal decidua, we know very little about Hoffbauer cells (HC) in fetal chorionic villi. In a study by Yang, no significant difference was found between the hypertensive and normotensive groups in terms of HC diameter. In the continuation of our study, CD14, CD68, and CD163 immunostaining were applied to placental sections and they emphasized that HC in the hypertensive group was weak in terms of immunostaining when compared to the control group [13].

In another study by Zhenghua et al., changes in the number and appearance of HC were reported to be associated with different pregnancy complications [14]. The results of our study are also supported by Demir et al and Kondi-Pafiti et al. These authors pointed out that there is a significant increase in the number of HC in pathological placentas such as gestational diabetes mellitus and intrauterine growth retardation [15-16].

Jones et al. [17] reported severe degenerative changes in the ultrastructural analysis of syncytial cell nuclei, such as pyknosis, peripheral chromatin condensation, and fusion of nuclear membranes. These morphological changes are similar to those of apoptosis, known as programmed cell death [18]. In similar studies, the increase in the number of apoptotic nuclei in trophoblasts of patients with preeclampsia has been pointed out [19-21]. Rath et al. [22] reported that trophoblastic basement membrane thickening was associated with preeclampsia and HELLP. Increased syncytiotrophoblasts in the HELLP placentas cause lesser absorption from the maternal blood as a result of a significant loss of microvilli, and thus the malnutrition of the fetus. In our study, we observed increasingly larger vacuoles and decreased pinocytic vesicles in the cytoplasm of syncytiotrophoblast cells of the HELLP group, suggesting decreased transport characteristics of syncytiotrophoblasts. Dilatations in the rough endoplasmic reticulum [ER] cisternae, which are observed in these cases, and low electron density accumulation in them are responsible for the basement membrane thickening. Therefore, we emphasized that thickening basal membranes negatively affects the placental barrier function.

In a study by Brunoria et al. [23], they emphasized that smooth ER and rough ER cisterns are very dilated in syncytiotrophoblast cells of HELLP. This event is parallel to the results of our study. Especially in some chorion villi of HELLP placentas, we observed excessive proliferation of cytotrophoblasts and their invasion into stroma as common epithelial mass. Thus, since the stroma is confined in an extremely narrow central region, we thought of considerably reduced or even completely lost placental barrier function in these villi. HELLP syndrome is a systemic disease manifested by cytotrophoblast invasion deficiency or maternal endothelial dysfunction. Roberts et al. [24] showed that the most important factor in this disease is excessive maternal systemic inflammation or uteroplacental hypoxia against pregnancy.

The number of HC in the HELLP group of our study showed a significant increase compared to the control group, and this increase was statistically significant. These results of our study showed a

correlation with the results of the study conducted by Evsen et al. [25]. These researchers emphasized that the number of Hoffbauer cells in the placental villi of patients with HELLP syndrome showed a significant increase compared to the normotensive group. Evsen et al. indicated a significant increase in Hoffbauer cell count in HELLP syndrome. They suggested that this increase may be related to increased inflammation or adaptation mechanism in the fetal placenta. In the immunohistochemical results of our study, compared with the control group, we can say that there was a significant increase in Hoffbauer cell count in the HELLP group placentas. Hoffbauer cells are fetal tissue macrophages in the chorionic villus stroma of the human placenta. This cell population constitutes 40% of the villous stroma and continues to exist during pregnancy. Hoffbauer cells secrete prostaglandin E2 (PGE2) and thromboxane A2 (PXA2). There are publications indicating that the amount of PGE2 and TXA2 released by Hoffbauer cells in a low-oxygen culture medium is reduced [26].

In conclusion, a significant increase in placental Hoffbauer cells and syncytiotrophoblast cell counts as well as several ultrastructural changes were observed in the HELLP group compared to the control group.

Declarations

Ethical statement: Ethical approval was obtained from the Dicle University Faculty of Medicine non-invasive clinical research ethics committee with a record number:456. This study was performed in accordance with Helsinki Declarations. Financial support was funded by the Dicle University Scientific Research Platform (record number: 13-TF-89).

Conflict of interest:

The authors declare there is no conflict of interest.

Authors' Contributions:

Writing - Original draft preparation: Y.N., Ş.N., F.A.
Methodology: U.Ş., S.T.
Formal analysis: Y.N.
Writing: Y.N., Ş.N.
Conceptualization: Y.N., E.S.
Methodology: Y.N., S.E.
Investigation: S.T., F.A., E.D.
Resources: E.D.
Investigation: Y.N., F.A.
All authors read and approved the final manuscript.

References

[1] C. Grigoriadis *et al.*, "Hofbauer cells morphology and density in placentas from normal and pathological gestations," (in eng), *Rev Bras Ginecol Obstet,* vol. 35, no. 9, pp. 407-12, Sep 2013, doi: 10.1590/s0100-72032013000900005.

[2] M. T. Vinnars, E. Rindsjö, S. Ghazi, A. Sundberg, and N. Papadogiannakis, "The number of CD68(+) (Hofbauer) cells is decreased in placentas with chorioamnionitis and with advancing gestational age," (in eng), *Pediatr Dev Pathol,* vol. 13, no. 4, pp. 300-4, Jul-Aug 2010, doi: 10.2350/09-03-0632-oa.1.

[3] A. Kondi-Pafiti, C. Grigoriadis, D. Samiotaki, A. Filippidou-Giannopoulou, C. Kleanthis, and D. Hassiakos, "Immunohistochemical study of inhibin A and B expression in placentas from normal and pathological gestations," (in eng), *Clin Exp Obstet Gynecol,* vol. 40, no. 1, pp. 109-12, 2013.

[4] B. F. King, "Ultrastructural differentiation of stromal and vascular components in early macaque placental villi," (in eng), *Am J Anat*, vol. 178, no. 1, pp. 30-44, Jan 1987, doi: 10.1002/aja.1001780105.

[5] E. Gokalp-Ozkorkmaz *et al.*, "Examination of Bcl-2 and Bax Protein Levels for Determining the Apoptotic Changes in Placentas with Gestational Diabetes and Preeclampsia," *Proceedings*, vol. 2, no. 25, p. 1548, 2018. [Online]. Available: https://www.mdpi.com/2504-3900/2/25/1548.

[6] M. S. Suresh, "HELLP SYNDROME: An Anesthesiologist's Perspective," *Anesthesiology Clinics of North America*, vol. 16, no. 2, pp. 331-348, 1998/06/01/ 1998, doi: https://doi.org/10.1016/S0889-8537(05)70025-0.

[7] C. J. Jones, R. H. Choudhury, and J. D. Aplin, "Functional changes in Hofbauer cell glycobiology during human pregnancy," (in eng), *Placenta*, vol. 36, no. 10, pp. 1130-7, Oct 2015, doi: 10.1016/j.placenta.2015.07.131.

[8] Y. Seval, E. T. Korgun, and R. Demir, "Hofbauer cells in early human placenta: possible implications in vasculogenesis and angiogenesis," (in eng), *Placenta*, vol. 28, no. 8-9, pp. 841-5, Aug-Sep 2007, doi: 10.1016/j.placenta.2007.01.010.

[9] F. Asir, E. Deveci, E. G. Ozkorkmaz, F. Şahin, and I. S. Ermiş, "The Effect of Momordica charantia Ovarian Ischemia-Reperfusion," *JBM*, vol. 9, no. 12, pp. 8-14, 2021.

[10] A. S. Laganà, A. Favilli, O. Triolo, R. Granese, and S. Gerli, "Early serum markers of pre-eclampsia: are we stepping forward?," (in eng), *J Matern Fetal Neonatal Med*, vol. 29, no. 18, pp. 3019-23, Sep 2016, doi: 10.3109/14767058.2015.1113522.

[11] S. S. Karaşin and T. Çift, "The Role of Ischemia-modified Albumin as a Biomarker in Preeclampsia," (in eng), *Rev Bras Ginecol Obstet*, vol. 42, no. 3, pp. 133-139, Mar 2020, doi: 10.1055/s-0040-1709662.

[12] S. W. Yang *et al.*, "DC-SIGN expression in Hofbauer cells may play an important role in immune tolerance in fetal chorionic villi during the development of preeclampsia," (in eng), *J Reprod Immunol*, vol. 124, pp. 30-37, Nov 2017, doi: 10.1016/j.jri.2017.09.012.

[13] Z. Tang, V. M. Abrahams, G. Mor, and S. Guller, "Placental Hofbauer cells and complications of pregnancy," (in eng), *Ann N Y Acad Sci*, vol. 1221, pp. 103-8, Mar 2011, doi: 10.1111/j.1749-6632.2010.05932.x.

[14] R. Demir and T. Erbengi, "Some new findings about Hofbauer cells in the chorionic villi of the human placenta," (in eng), *Acta Anat (Basel)*, vol. 119, no. 1, pp. 18-26, 1984, doi: 10.1159/000145857.

[15] C. J. Jones and H. Fox, "Syncytial knots and intervillous bridges in the human placenta: an ultrastructural study," (in eng), *J Anat*, vol. 124, no. Pt 2, pp. 275-86, Nov 1977.

[16] J. F. Kerr, A. H. Wyllie, and A. R. Currie, "Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics," (in eng), *Br J Cancer*, vol. 26, no. 4, pp. 239-57, Aug 1972, doi: 10.1038/bjc.1972.33.

[17] S. C. Smith, P. N. Baker, and E. M. Symonds, "Increased placental apoptosis in intrauterine growth restriction," (in eng), *Am J Obstet Gynecol*, vol. 177, no. 6, pp. 1395-401, Dec 1997, doi: 10.1016/s0002-9378(97)70081-4.

[18] D. N. Leung, S. C. Smith, K. F. To, D. S. Sahota, and P. N. Baker, "Increased placental apoptosis in pregnancies complicated by preeclampsia," (in eng), *Am J Obstet Gynecol,* vol. 184, no. 6, pp. 1249-50, May 2001, doi: 10.1067/mob.2001.112906.

[19] A. D. Allaire, K. A. Ballenger, S. R. Wells, M. J. McMahon, and B. A. Lessey, "Placental apoptosis in preeclampsia," (in eng), *Obstet Gynecol,* vol. 96, no. 2, pp. 271-6, Aug 2000, doi: 10.1016/s0029-7844(00)00895-4.

[20] S. Sreenivasan and S. Khare, "The effects of passive smoking on the human placenta: A gross and microscopic study," *Journal of the Anatomical Society of India,* Original Article vol. 68, no. 1, pp. 34-38, January 1, 2019 2019, doi: 10.4103/jasi.Jasi_5_19.

[21] I. de Luca Brunori *et al.*, "Placental barrier breakage in preeclampsia: ultrastructural evidence," (in eng), *Eur J Obstet Gynecol Reprod Biol,* vol. 118, no. 2, pp. 182-9, Feb 1 2005, doi: 10.1016/j.ejogrb.2004.04.024.

[22] J. M. Roberts, R. N. Taylor, and A. Goldfien, "Clinical and biochemical evidence of endothelial cell dysfunction in the pregnancy syndrome preeclampsia," (in eng), *Am J Hypertens,* vol. 4, no. 8, pp. 700-8, Aug 1991, doi: 10.1093/ajh/4.8.700.

[23] M. S. Evsen *et al.*, "Human placental macrophages (Hofbauer cells) in severe preeclampsia complicated by HELLP syndrome: immunohistochemistry of chorionic villi," (in eng), *Anal Quant Cytopathol Histpathol,* vol. 35, no. 5, pp. 283-8, Oct 2013.

[24] B. Wetzka, D. E. Clark, D. S. Charnock-Jones, H. P. Zahradnik, and S. K. Smith, "Isolation of macrophages (Hofbauer cells) from human term placenta and their prostaglandin E2 and thromboxane production," (in eng), *Hum Reprod,* vol. 12, no. 4, pp. 847-52, Apr 1997, doi: 10.1093/humrep/12.4.847.