

Full Length Research Paper

Immunohistochemical evaluation of iron accumulation in term placenta of preeclamptic patients

Yasemin Nasir¹, Yusuf Nergiz¹, Ayfer Aktaş^{1*} and Nurten Akdeniz²

¹Departments of Histology and Embryology, Medical Faculty, Dicle University, 21280 Diyarbakir, Turkey.

²Department of Obstetrics and Gynecology, Medical Faculty, Dicle University, 21280 Diyarbakir, Turkey.

Accepted 17 June, 2011

Preeclampsia is a disease which involves hypertension and multisystem, it effects approximately 2 to 8% of all pregnancies and is a significant cause of maternal and newborn mortality and morbidity. It develops in the placenta and its pathogenesis is associated with placental abnormalities. Classical immunohistochemical studies on placenta have shown that there is a linear increase in iron storage in the placenta in the first half of a normal pregnancy, however, these stocks are decreased in normal 3rd trimester placenta. Iron accumulation in term placentas of preeclamptic and normal pregnancies were evaluated in this study. Ferritin immunostaining was observed to be more intense in preeclamptic group than in the control group, especially in Hofbauer cells, subtrophoblastic areas of stem villous, perivascular stroma and villous stroma. Statistical analysis of the data was performed using SPSS software. Mann Whitney U test was used in the analysis and P values below 0.05 were statistically significant. In this study, iron accumulations in normotensif and preeclamptic placentas were compared.

Key words: Preeclampsia, placenta, immunohistochemistry, iron accumulation.

INTRODUCTION

Preeclampsia is a systemic disorder associated with the placenta. Its etiopathogenesis is still not completely defined and there are various hypotheses about its cause (Bulmer, 1992; Burrows et al., 1994).

Low placentation plays an important role in the etiology of preeclampsia. Placental perfusion deficiency and abnormal trophoblast invasion are shown to cause preeclampsia (Roberts et al., 2001). Increased frequency of preeclampsia in cases of disorder in the formation of blocking antibodies against antigenic regions on placenta or where these antibodies are insufficient leads to the opinion that preeclampsia might be caused by a disorder in the immune system. Increased incidence of preeclampsia in cases without immunization in a previous pregnancy, such as first pregnancies, or in multiple pregnancies, molar pregnancies and in multipar females gotten pregnant by a new partner support this theory.

Endothelial dysfunction is important in pathogenesis of preeclampsia (Dietl, 2000). Basis of preeclampsia/eclampsia pathophysiology is vasospasm, which creates a resistance against blood flow and causes development of arterial hypertension. Harmful effect of vasospasm itself on vessels is also known. Angiotensin II also causes injuries in endothelial cells. All these changes cause endothelial cell damage, leading to infusion and accumulation of blood ingredients, including thrombocyte and fibrin inside the subendothelial region. Eventually, local hypoxia, hemorrhage, necrosis and fibrin accumulation occurs (Cunningham, 2001).

Healthy continuation and successful ending of pregnancy necessitates adequate development of placental circulation. Thrombus formation in placental circulation can cause preeclampsia, intrauterine underdevelopment and intrauterine death (Brenner et al., 1997). Studies showed that preeclampsia and eclampsia might be hereditary (Cooper and Liston, 1979).

This study aimed to determine placental iron accumulation in term placentas of preeclamptic and normotensive pregnancies of 36 weeks or above by immunohisto-

*Corresponding author. E-mail: fuldic@gmail.com. Fax: + 90 412 2488440.

chemical methods.

MATERIALS AND METHODS

Tissues patient population was composed of pregnant females that were registered in Dicle University, Faculty of Medicine, Obstetrics and Gynecology Department. Human material of the study was approved by Dicle University Faculty of Medicine human researches board of ethics.

Placentas were fixed in 10% neutral formalin immediately after birth. Taking morphological attributes of placentas into account, approximately 1 x 1 cm tissue samples were collected from fetal and maternal sides from four different regions; maternal placenta central section (MC), maternal placenta peripheric section (MP), fetal placenta central section (FC) and fetal placenta peripheric section (FP). Basis of central sampling was the center of placental disc and sampling was made from 3 cm away from the center. Peripheric samples was taken from 2 cm of the inside of the placental disc.

Antibodies and staining procedure

The tissue samples were immediately frozen in liquid nitrogen at -196°C and were kept in a -30°C deep freeze until use. Seven micrometer thick serial sections were cut using a cryostat, and these sections were mounted on gelation covered microscopic slides. After drying at room temperature, the samples were kept in humidity free containers with silica gel (Merc,1.01925) until immunoreactivity was performed as previously described by Khatun et al. (2003). Ferritin antibody (Gene Tex, Inc.GTX Rabbit anti-mouse) was used as the primary antibody. Biotimylated goat anti-polyvalent (ScyTec laboratories) was used as the secondary antibody.

Control staining was performed, omitting the primary antibody step. Sections were examined and photographed by Olympus BH2 light microscope.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) 14.0 was the software used for statistical calculations. Demographic data, clinical findings and maternal blood levels of preeclamptic and normal pregnancy groups were statistically compared using Mann Whitney U test. Intergroup significance of villous involvement, percentages of preeclamptic and normal pregnancy placentas that were applied with ferritin immuno-histo-chemistry by immunoperoxidase method were evaluated using Mann Whitney U test (Yildiz et al., 2001).

Values were presented as "mean \pm standard deviation". In all evaluations, P values below 0.05 were regarded as significant.

RESULTS

Control group immunohistochemical results

In each of the MC, MP, FC and FP sections of placentas, ferritin immunoreactivity was observed. Ferritin accumulations showed a homogeneous distribution in all layers of maternal and fetal surfaces of the placenta.

Ferritin accumulation in villous was observed predominantly in syncytial layer, subtrophoblastic region, villous stroma and Hofbauer cell (Figure 1). In some

sections, despite some sectional differences in villous endothelial vein and perivascular stroma (Figure 2), presence of various ferritin intensity was shown (Figure 4).

In contrast to others, FC sections showed more intense ferritin accumulation in villous stroma (Figure 3). Ferritin immunoreactivity, with some individual differences, was found to be weaker in terms of villous staining rates in control group MC, MP, FC and FP sections as compared to those of preeclamptic group. Staining was not seen in negative controls (Figure 5).

Preeclampsia group immunohistochemical results

In preeclampsia placentas, Hofbauer cells in higher numbers, showed more ferritin immunoreactivity than those in control placentas. Hofbauer cells were observed most in FC sections (Figure 7). In preeclampsia, ferritin was seen to increase in central zones of the placenta, rather than in peripheric sections. Ferritin accumulation was seen most in FC sections (Figure 6), than in the MC sections (Figure 7) of preeclampsia group. Ferritin immunoreactivity in stem villous, similar to terminal villi, was seen intensely in subtrophoblastic region, perivascular stroma and villous stroma. The least staining was visualized in MP sections (Figure 8) and FP sections (Figure 9), respectively.

In sections where ferritin accumulation was seen in more villi and more intensely, atrophic vill, syncytial knots and cytotrophoblast proliferation was also more (Figure 6). In most sections, villous syncytial layer continuity was disturbed and syncytiotrophoblasts were unavailable. Major finding accompanying loss of syncytiotrophoblasts was cytotrophoblast proliferation and intervillous bridges and a labyrinth-like view in preeclamptic sections were observed (Figure 6 to 7). While ferritin immunoreactivity in fetal vascular endothelium was seen in a few sections, the negative controls did not show any staining (Figure 10).

Ferritin staining of placental villi in MC sections were 62.00 ± 9.933 in the controls and 78.00 ± 12.60 in the preeclampsia group. In FC sections, they were 48.50 ± 7.215 in the controls and 87.00 ± 6.366 in the preeclampsia group. As a consequence, a statistically significant difference was detected between groups ($p < 0.05$). In terms of ferritin immunoreactivity in MP and FP sections, a statistically significant difference was not found between preeclampsia and the control groups ($p > 0.05$).

Statistical analysis of immunohistochemical results

Results of the immunohistochemical staining of the groups is given in Table 1 and values of ferritin-staining villi are shown in graphic 1.

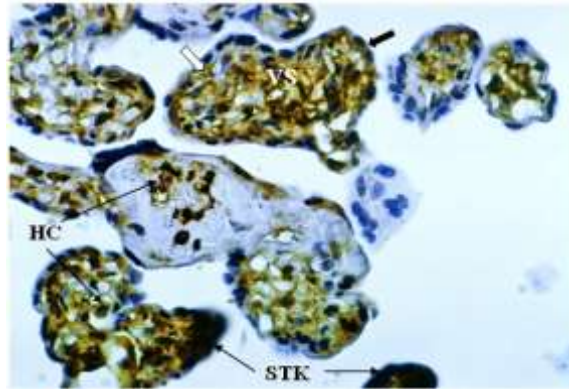


Figure 1. Ferritin immunostaining in the control group maternal placenta central zone. Positive ferritin immunoreactivity viewed in syncytial layer (black arrow), subtrophoblastic region (white arrow), villous stroma (VS) and Hofbauer cells (HC), syncytial knot (STK), syncytial knot. Original magnification; 160x.

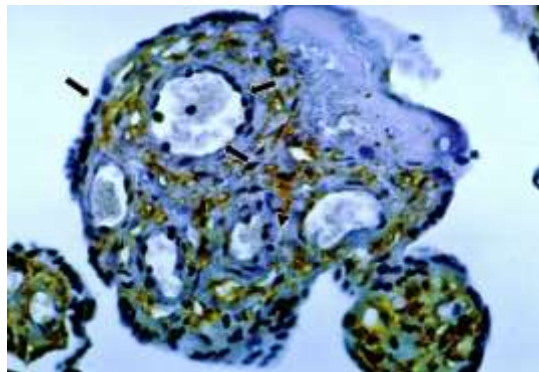


Figure 2. Ferritin immunostaining in the control group maternal placenta peripheric zone. Ferritin immunoreactivity of different intensity viewed in villous vein endothelium (arrow head) and perivascular stroma (arrows). Original magnification; 160x.

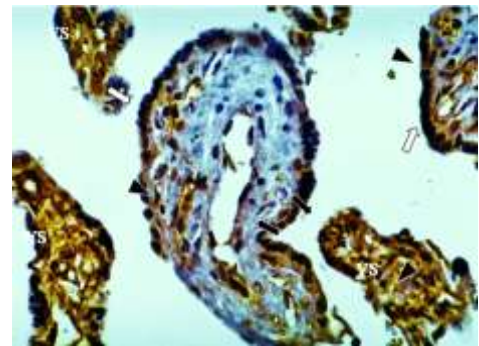


Figure 4. Ferritin immunostaining in the control group fetal placenta peripheric zone. Ferritin immunoreactivity of different intensity viewed in syncytial layer (black arrows), subtrophoblastic region (white arrows), villous stroma (VS) and Hofbauer cells (arrow heads). Original magnification; 160x.

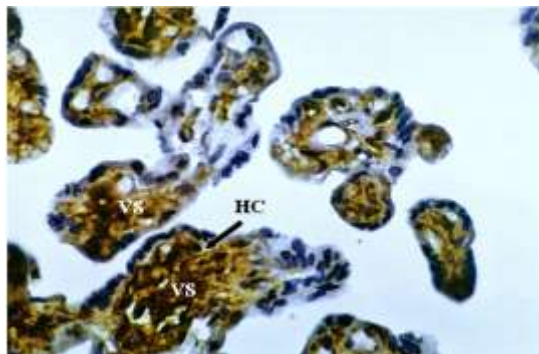


Figure 3. Ferritin immunostaining in the control group fetal placenta central zone. Intense ferritin immunoreactivity viewed in Hofbauer cells (HC) and villous stroma (VS). Original magnification; 160x.

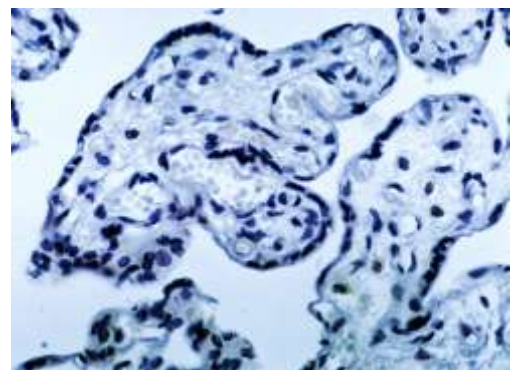


Figure 5. Negative control section of the control group maternal placenta peripheric zone. Original magnification; 160x.

DISCUSSION

Ferritin is a protein that is found as a complex with iron and is predominately present in liver, spleen and bone marrow as a major iron source. Previously, ferritin was regarded only as a storage protein that transfer circulation by hepatocyte decomposition. However, today it is known to be a ferritin pool in the circulation (Kaneshige, 1981). Many studies in literature have reported increasing ferritin levels in preeclampsia with increasing severity and the underlying causes are discussed (Gitlin, 1992).

Raman et al. (1992) did not detect change in liver enzymes and hemoconcentration in their study, thus, concluding that liver damage and hemodynamic changes play minor role in hyperferritinemia, and stressed on the possible role of placental ferritin.

In our study, histologic investigation of control group placentas resulted in normal view of villous trophoblastic layer, villous stroma and fetal vascular structures, whereas most common histologic findings in preeclamptic group placentas were increased in villous syncytial knot, increased in fetal capillary number-volume and increased in fibrinoid accumulation, Hofbauer cells and atrophic villi (Figure 6). It is reported that in preeclampsia, many cytokines are secreted from Hofbauer cells that stimulate vasculogenesis and angiogenesis (Rinehart et al., 1999; Numasaki et al., 2003).

In our study, Hofbauer cells were found to be in larger numbers in preeclamptic sections. Both early and recent studies are present in literature supporting this finding (Fox, 1967). Seval et al. (2007), showed a correlation between number of Hofbauer cells in placental villous and number of vascular structures in preeclampsia (Seval et al., 2007). Studies showing secretion of IL-71, proangiogenic molecule that induces neovascularization by Hofbauer cells in term placenta are present (Numasaki et al., 2003; Pongcharoen et al., 2007). In the light of literature information, increase of number and volume of fetal capillary in preeclamptic placentas, along with increase in the number of Hofbauer cells, in our study was explained by increase of cytokines, especially VEGF and IL-17, secreted from Hofbauer cells.

Transferrin receptors (TfRs) have been shown immunohistochemically in both normotensif and preeclampsia, in villi syncytiotrophoblasts, fetal vascular endothelial cells and amniotic membranes (Khatun et al., 2003). In our study also, the presence of ferritin in fetal vascular endothelium was shown in some of the preeclamptic placenta cross sections (Figure 6). This finding supports that TfRs are placed in vascular endothelium.

Khatun et al. (2003) investigated TfRs immunohistochemically in their study on syncytiotrophoblasts in normal and atrophic villi in preeclamptic placentas. In that study, atrophic villi/total villi ratio in preeclamptic group was found to be substantially higher than that in normal pregnancy placentas. In correlation, atrophic villi were viewed in higher numbers in preeclamptic placenta sec-

tions in our study (Figure 6).

Khatun et al. (2003) showed strong positive TfR immunoreactivity in the syncytiotrophoblastic region in both normal and atrophic villous in normal pregnancy placentas. In preeclamptic placentas, on the other hand, very low TfR immunoreactivity and very low rate of positive cell staining were detected. Immuno-histochemical staining by anti-HCG antibody, performed with the control, resulted in strong positive immunostaining in syncytiotrophoblastic region of the normal and atrophic villous in both preeclampsia and control groups. While strong TfR immunoreactivity was detected in extravillous syncytiotrophoblasts, villous vessel endothelial cells and amniotic membranes of decidua, very low staining was shown in these regions of the preeclamptic placentas. Consequently, decrease in TfR production of syncytiotrophoblasts in preeclampsia was shown. Babies of preeclamptic mothers were also shown to be born an average of 6 weeks earlier than the control group. This increased prematurity was explained by decrease of the placental TfR in preeclampsia (Khatun et al., 2003; Van Dijk et al., 1993).

Increased cellular iron concentration resulting from decrease in TfR explains increase in total serum iron and ferritin in preeclampsia and increase in cellular iron concentration is also seen in placental tissue (Rayman et al., 2002). Khatun et al. (2003) described ferritin as the most prominent indicator of decrease in TfRs and of cellular iron accumulation in preeclampsia.

In our study, in the cross sections where ferritin immunohistochemistry was applied, iron storage was determined to be significantly higher in preeclamptic group than in the controls. Our findings indicated increased cellular iron storage in preeclampsia and this resulted from decrease in TfR production in syncytiotrophoblasts, supporting the study of Khatun et al. (2003).

Decrease in TfRs also explains increase of serum ferritin levels of preeclamptic females. In parallel, only hematologic parameter that significantly increased in the preeclampsia group was found to be maternal serum ferritin in our study. In our study, the control group maternal Tf and total iron binding capacity (TIBC) was higher and this was concluded to be a physiological increase in normal pregnancy. Statistically significant lower maternal Tf and TIBC in preeclampsia group is regarded as a clear indication that preeclampsia disturbs maternal adaptation mechanisms and physiology in pregnancy.

In our study, increased maternal serum ferritin in preeclampsia group and higher ferritin immunoreactivity in preeclampsia group than in the controls, shown by placenta immunohistochemistry can be explained in two ways: either decrease of TfRs in placental and other tissues, or increase of ferritin as a compensatory mechanism of antioxidant system. Results of literature studies on preeclampsia, antioxidant systems, TfRs, ferritin and placenta are correlated with our results.

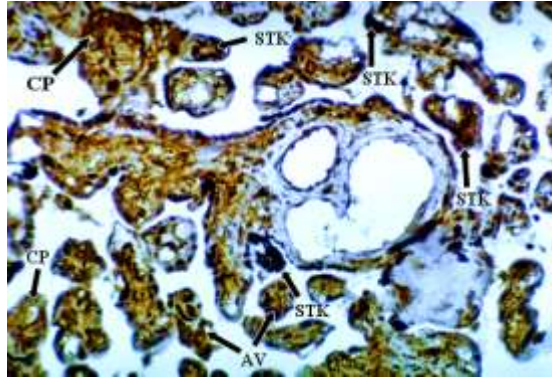


Figure 6. Ferritin immunostaining of preeclamptic group fetal placenta central zone. Many atrophic villi with intense ferritin accumulation, increased syncytial knots and cytotrophoblast proliferations were seen. Atrophic villous (AV), syncytial knot (STK), cytotrophoblast proliferation (CP). Original magnification; 80x.

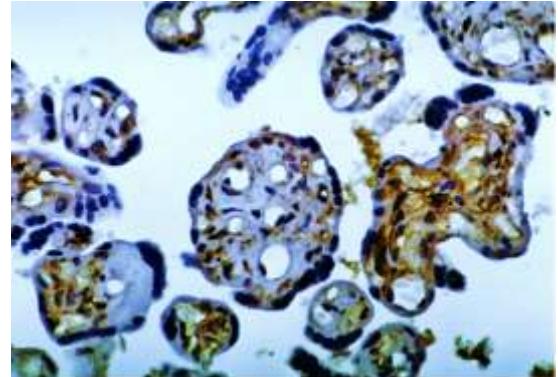


Figure 9. Ferritin immunostaining of preeclamptic group fetal placenta peripheral zone. Heterogeneity and lower intensity in villi ferritin immunoreactivity are seen as compared to FC, MC and MP sections. Original magnification; 160x.

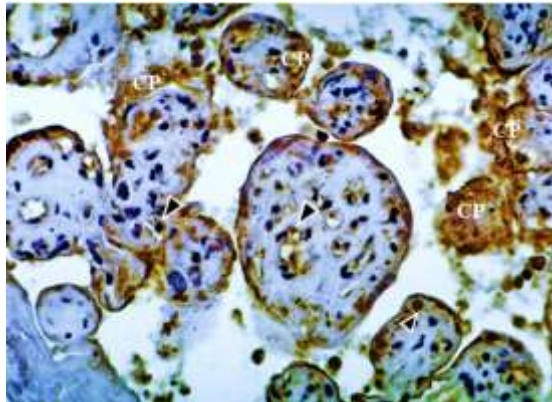


Figure 7. Ferritin immunostaining of preeclamptic group maternal placenta central zone. Note cytotrophoblast proliferation in villi and immunostaining in Hofbauer cells (arrow heads). Original magnification; 160x.

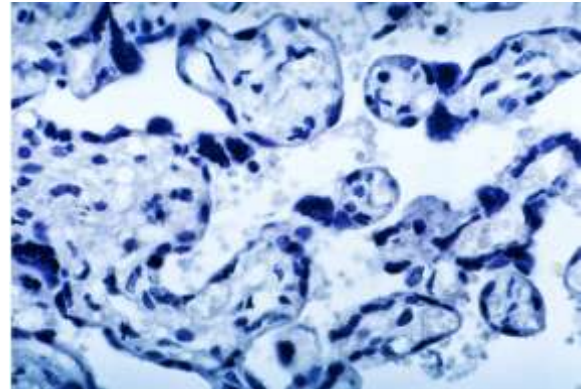


Figure 10. Negative control section collected from maternal peripheral zone of preeclamptic placenta group. Original magnification; 160x.

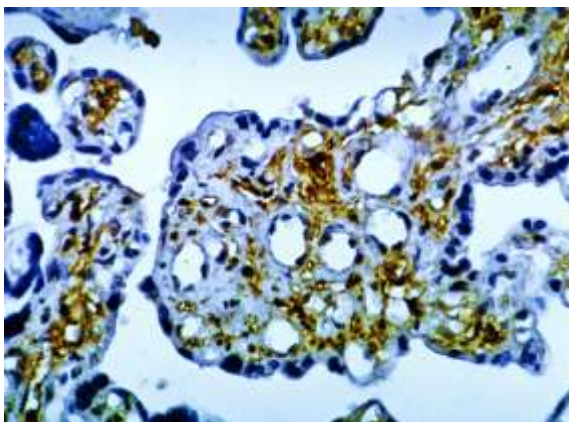


Figure 8. Ferritin immunostaining of preeclamptic group maternal placenta peripheral zone. Less intense ferritin immunoreactivity in FC and MC sections. Original magnification; 160x.

The fact that increase of ferritin accumulation in preeclampsia group was higher in the central cross sections of the placenta in our study presented a new dimension to the fetal and maternal placenta classification being taken as a basis in placental researches. This situation suggests that placental kinetics, transportation, storage and endocrine functions differ in central and peripheral sections of the placenta, just as they do in fetal and maternal sides of it. Also, the opinion arises that central regions of the placenta are affected more in preeclampsia. However, further information and studies are necessary on this aspect.

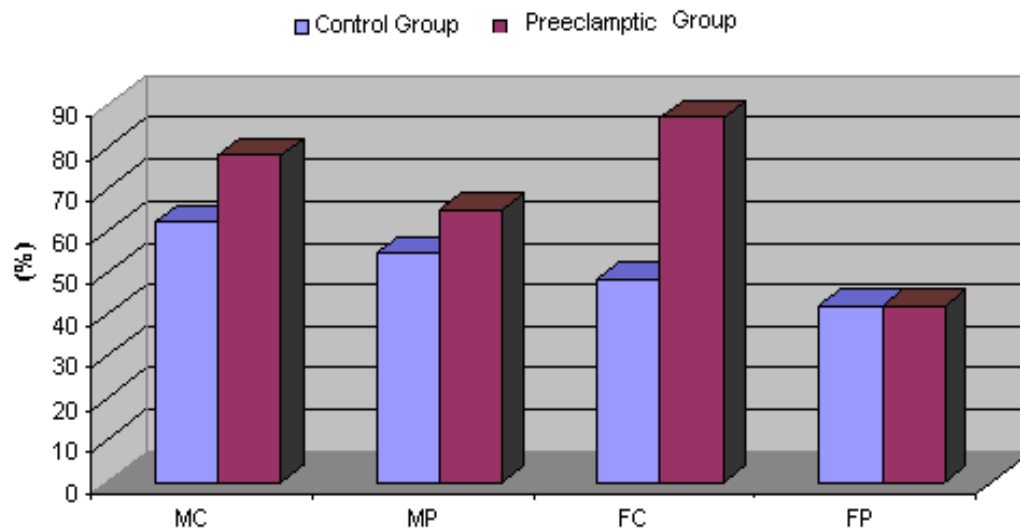
Conclusion

Placental immunohistochemical studies are important in the clarification of preeclampsia etiopathogenesis, and further studies with broader scope are necessary in this

Table 1. Results of ferritin-staining of placental villi.

Placental villi section	Preeclamptic group (n = 20)		Control group (n = 10)		Significance
	Mean	SD	Mean	SD	
MC Section	78.00	12.60	62.00	9.933	z: 3.061 p: 0.001*
MP Section	65.00	13.39	54.50	6.450	z: 1.916 p: 0.055
FC Section	87.00	6.366	48.50	7.215	z: 4.399 p: 0.000*
FP Section	42.00	7.462	42.00	7.513	z: 0.132 p: 0.914

z: Mann Whitney U test, *p<0.05 statistically significant.



Graph 1. Histogram showing ferritin-staining villi percentage. MC, Maternal placenta central section; MP, maternal placenta peripheric section; FC, fetal placenta central section; FP, fetal placenta peripheric section.

field.

REFERENCES

- Brenner B, Mandel H, Lanir N, Younis J, Rothbart H, Ohel G, Blumenfeld Z. (1997). Activated protein C resistance can be associated with recurrent fetal loss. *Br. J. Haematol.* 97: 551-554.
- Bulmer JN (1992). Immune aspects of pathology of the placental bed contributing to pregnancy pathology. *Balliere's Clinic.Obstet. and Gynaecol.* 6: 461-487.
- Burrows TD, King A, Loke YW (1994). Expression of adhesion molecules by endovascular trophoblast and decidual endothelial cells: Implications for vascular invasion during implantation. *Placenta*, 15: 21-33.
- Cooper DW, Liston WA (1979). Genetic control of severe preeclampsia. *J. Med. Genet.* 16: 409.
- Cunningham FG (2001). Hypertensive disorders in pregnancy. 21st edition, The McGraw-Hill Companies, USA.
- Dietl J (2000). The pathogenesis of pre-eclampsia: New aspects. *J. Perinat. Med.* 28: 464-471.
- Fox H (1967) The incidence and significance of Hofbauer cells in the mature human placenta. *J. Pathol. Bacterio.* 93: 710-717.
- Gitlin N (1992). Liver disease in pregnancy. 3rd ed, WB Saunders Company Ltd, London,
- Kaneshige E (1981). Serum ferritin as an assesment of iron stores and other hematologic parameters during pregnancy. *Obstet. Gynecol.* 57: 238-241.
- Khatun R, Wu Y, Kanenishi K, Ueno M, Tanaka S, Hata T, Sakamoto H (2003). Immunohistochemical study of transferrin receptor expression in the placenta of pre-eclamptic pregnancy. *Placenta*, 24: 870-876.
- Numasaki M, Fukushi J, Ono M, Narula SK, Zavodny PJ, Kudo T (2003). Interleukin-17 promotes angiogenesis and tumor growth. *Blood.* 101: 2620-27.
- Pongcharoen S, Somran J, Sritippayawan S, Niumsup P, Chanchan P, Butkhamchot P, Tatiwat P, Kunngurn S, Searle RF. (2007). Interleukin-17 Expression in the human placenta. *Placenta*, 28: 59-63.
- Raman L, Pawashe AB, Yasodhara P (1992). Hyperferritinemia in pregnancy induced hypertension and eclampsia. *J. Postgrad. Med.* 38: 65-67.
- Rayman MP, Barlis J, Evans RW, Redman CW, King LJ (2002). Abnormal iron parameters in the pregnancy syndrome

- preeclampsia. *Am. J. Obstet. Gynecol.* 187: 412-418.
- Rinehart BK, Terrone DA, Lagoo-Deenadayalan S, Barber WH, Hale EA, Martin JN Jr, Bennett WA (1999). Expression of the placental cytokines tumor necrosis factor α , interleukin 1 β , and interleukin 10 is increased in preeclampsia. *Am. J. Obstet. Gynecol.* 181: 915-20.
- Roberts JM, Cooper DW (2001). Pathogenesis and genetics of preeclampsia. *Lancet*, 357: 53-56.
- Seval Y, Korgun ET, Demir R (2007). Hofbauer cells in early human placenta: possible implications in vasculogenesis and angiogenesis. *Placenta*, 28: 841-845.
- Van Dijk JP, van der Zande FG, Kroos MJ, Starreveld JS, van Eijk HG (1993). Number and affinity of transferrin receptors at the placental microvillous plasma membrane of the guinea pig: Influence of gestational age and degree of transferrin glycan chain complexity. *J. Dev. Physiol.* 19: 221-226.
- Yıldız L, Aydın O, Kurt K, Barış S, Karagöz F, Kandemir B (2001). Placental iron deposits. The meaning of molar pregnancy and fetal abnormalities. *The Turki. J. Pathol.* 17(1-2): 11-13.