Placental apoptosis in preeclampsia, intrauterine growth retardation, and HELLP syndrome: An immunohistochemical study with caspase-3 and bcl-2

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Summary

Objective: To examine the placental expression of caspase-3 and bcl-2 in pregnancies complicated by preeclampsia, IUGR, and HELLP syndrome. *Materials and Methods:* A prospective case-control study was conducted on 50 pregnant women between December 2006 and August 2007 at Dr. Zekai Tahir Burak Women Health Research and Education Hospital, Ankara, Turkey. Placental tissue samples were obtained from 15 pregnancies complicated by preeclampsia, 15 pregnancies with normotensive IUGR, five pregnancies with HELLP syndrome, and 15 gestational age-matched normotensive pregnancies without intrauterine infection as a control group. The placental expression of caspase-3 and bcl-2 has been investigated by immunohistochemical staining. *Results:* Caspase-3 immunostaining score was significantly higher in each group when compared with the control group ($p = 0.002$). However there was no statistically signifant difference with bcl-2 immunostaining in each group when compared with the control group. *Conclusions:* Apoptotic marker caspase-3 is significantly increased in the villous trophoblasts of patients with preeclampsia, HELLP syndrome, and IUGR indicating increased placental apoptosis.

Key words: Caspase-3; Bcl-2; Apoptosis; Preeclampsia; IUGR; HELLP syndrome.

Introduction

Hypertensive pregnancy disorders like preeclampsia, hemolysis, elevated liver enzymes, and low platelet (HELLP) syndrome, as well as intrauterine growth retardation (IUGR) are still some of the most common causes of maternal-fetal morbidity and mortality [1].

Although the etiology of these pregnancy disorders remains unclear, it is generally believed that impairment of trophoblastic invasion plays a key role in the etiology.

Impaired/shallow interstitial trophoblastic invasion leads to maladaptation of uteroplacental arteries and then a placental factor is released into maternal circulation causing maternal endothelium damage [2, 3].

Apoptosis, a form of programmed cell death, of trophoblasts has been detected in normal human placentas [4, 5] and in placentas of preeclampsia, IUGR, and HELLP syndrome [6-8].

In preeclampsia or IUGR, changes in apoptosis regulation of villous and extravillous trophoblasts results in altered trophoblastic invasion and then shedding into maternal circulation causing clinical symptoms [3].

However, in the literature the assessment of placental apoptosis differs due to different techniques and methods of quantification [9, 10].

Molecular mechanisms leading to apoptosis are complex and include signal transduction pathways that trigger or inhibit apoptosis [11]. The trigger of apoptosis depends on the balance between pro- and anti-apoptotic proteins.

Caspase 3 is a member of the cysteine-aspartic acid protease (caspase) family and exists as a zymogen (procaspase) in almost all cells and is involved in the development of apoptotic cell death [11].

Bcl-2 is an anti-apoptotic protein expressed in the trophoblast layer of placental villi and high expression of bcl-2 in syncytiotrophoblasts would protect this key layer of placental villi from apoptosis [12].

As a result there is a controversy about the role of placental apoptosis in hypertensive pregnancy disorders and IUGR. Increased placental apoptosis [4, 13,14] and reduced placental apoptosis [6, 15] in hypertensive pregnancy disorders and IUGR have been reported in the literature.

In this study, the authors have investigated the expression of caspase-3 and bcl-2 in the placental tissue samples of pregnancies complicated with preeclampsia, HELLP syndrome, and IUGR.

Materials and Methods

This was a prospective case-control study which was conducted on 50 pregnant women between December 2006 and August 2007 in the Department of Obstetrics and Gynecology at Dr. Zekai Tahir Burak Women Health Research and Education Hospital, Ankara, Turkey.

Placental tissue samples were obtained from 15 pregnancies complicated by preeclampsia, 15 pregnancies with normotensive IUGR, five pregnancies with HELLP syndrome, and 15 gestational age-matched normotensive pregnancies without

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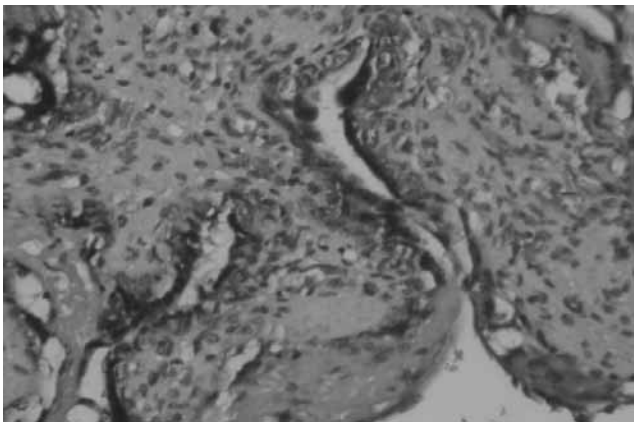
Fig. 1A



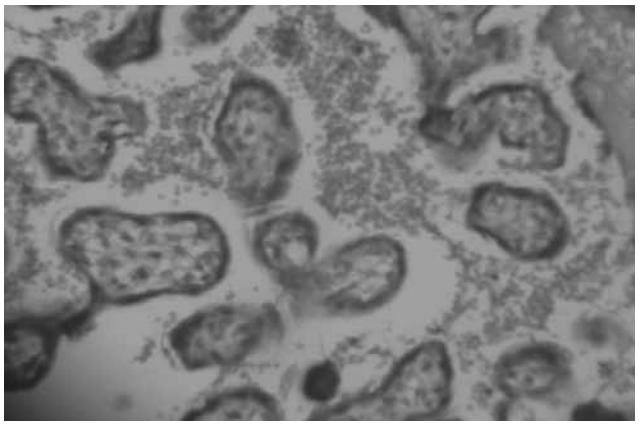


Fig. 1B

Figure 1. — Cytoplasmic staining with caspase-3 in placentas.
A) Weak and focal immunostaining (+1) in HELLP syndrome placenta of 27 weeks (200x).
B) Weak and diffuse immunostaining (+2) in IUGR placenta of 38 weeks (200x).

intrauterine infection as a control group. Written informed consent was obtained from all subjects and the study protocol was approved by the Institutional Ethics Committee.

Preeclampsia and HELLP syndrome were defined according to the National High Blood Pressure Education Program [16]. IUGR was defined as either an ultrasound (US) estimate of fetal weight or an US measurement of the fetal abdomen < 5th percentile for gestational age, confirmed at delivery (birthweight < 5th percentile for age and gender) and not associated with aneuploidy, structural anomalies, or congenital infection.

Placental samples were taken from both vaginal deliveries and cesarean sections. Analysis was performed in two placental samples from the central part of each placenta per patient. The samples were cut into small pieces (2 x 2 x 2 cm) and rapidly fixed in 10% formalin for 24 hours at room temperature. After fixation, samples were embedded in paraffin wax. From these blocks, three 5 μm sections were cut for each placental sample and mounted on slides. These slides were stained with hematoxylin and eosin and at least 20 fields were examined per slide. Different cell types in the placenta were identified under light microscope and selected for immunohistochemistry.

Immunohistochemistry for caspase-3 and bcl-2α was performed using a combination of the streptavidin-biotin-peroxidase method and microwave antigen retrieval on formaline-fixed paraffin embedded tissues. After deparaffinization, samples were treated with 10% hydrogen peroxidase in filtered water to block endogenous peroxidase activity. To attain antigen, slides were treated with 10 mmol/l citrate buffer (pH: 6. 8) for 10 min. After preincubation with Ultra V block (Lab Vision) for 20 min, samples were incubated with primary antibody for an hour at room temperature for caspase-3 (rabbit polyclonal antibody, CPP32.7 ml, Neomarkers) and bcl-2α Ab-1 (mouse monoclonal antibody, Clone 100/D5.7 ml, Neomarkers). The positive controls were lymphoid tissue for caspase-3 and lymphoma for bcl 2α. Negative control was achieved by the same way without application of the primary antibody.

The sections were examined by the pathologist at high power (x200) in a blinded design. Immunohistochemical evaluation was carried out in the epithelium of the trophoblastic cells. The percentage of positively stained area to the total area of villous trophoblasts in each section was calculated [17]. The staining score for caspase-3 and bcl-2 was labeled as '0' for no immunostaining, '1+' for weak and focal immunostaining, '2 +' for weak and diffuse immunostaining, '3+' for strong and diffuse immunostaining. The results of all four scores from each case were summarized to achieve the final immunostaining result.

Statistical analysis of the data was carried out by using the SPSS 13. To analyze the correlation between the scores from each group, the Kruskal-Wallis test was used. Differences were accepted as significant for $p < 0.05$.

Table 1. — *Clinical characteristics of each group*.

	Preeclampsia n = 15	Control n = 15	IUGR n = 15	HELLP n = 5
Age (yrs)	28.9 ± 8.6	27.1 ± 4.9	25.3 ± 5.6	29 ± 4.6
Parity	1.9 ± 0.7	2.1 ± 1.2	2.3 ± 1.4	2 ± 1
Gestational age(wks)	35.7 ± 3.4	35.1 ± 4.2	37.8 ± 2.3	26.5 ± 1.8¶
Fetal weight (g)	2070 ± 754	2633 ± 1078	2146 ± 415	650 ± 241†

Data are shown as means and standard deviations.
¶ $p < 0.05$.
† $p < 0.05$.

Table 2. — *Qualitative analysis of expression scores of caspase-3 and bcl-2 in each group*.

	Preeclampsia n = 15	Control n = 15	IUGR n = 15	HELLP n = 5	*p*
Caspase-3	1.0 ± 0.4*	0.5 ± 0.5	1.2 ± 0.4¶	1,2 ± 0,5†	0.002
Bcl-2	1.9 ± 0.8	2.3 ± 0.5	2.1 ± 1.0	2,2 ± 0,8	0.518

* Data are shown as means and standard deviations.
* The intensity of the immunostaining was graded '0' for no immunostaining, '1+' for weak and focal immunostaining, '2+' for weak and diffuse immunostaining, '3+' for strong and diffuse immunostaining.
* When compared with the control group *p = 0.015, ¶p = 0.001, †p = 0.015.

Results

Clinical characteristics of patients are summarized in Table 1. There were no significant differences in maternal age and parity between the study groups. However gestational age and fetal weight were significantly lower in the HELLP group than the control group ($p < 0.05$).

Immunohistochemical analysis of caspase- 3 and bcl- 2 in the placental tissue samples of patients are shown in Table 2. Examples of placental staining with caspase-3 and bcl-2 are shown in Figures 1 and 2, respectively.

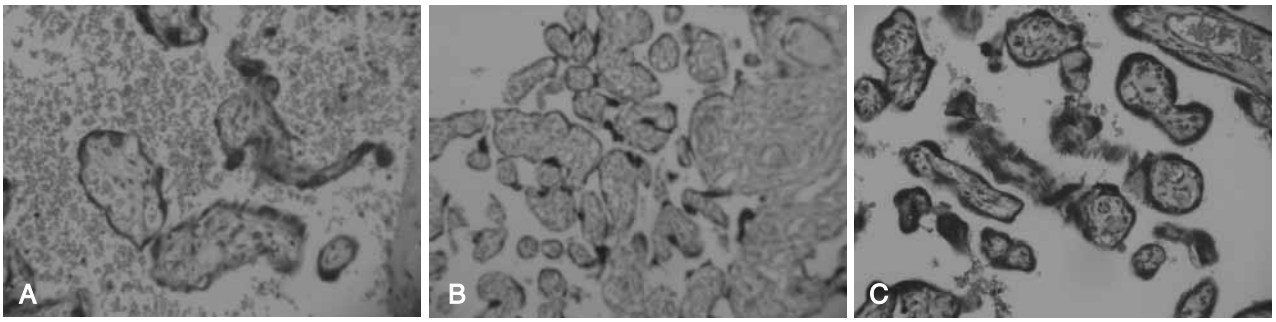


Figure 2. — Cytoplasmic staining with bcl-2 in placentas.
A) Weak and focal immunostaining (+1) in placenta of 28 weeks in preeclamptic group (200x).
B) Weak and diffuse immunostaining (+2) in placenta of 39 weeks in control group (200x).
C) Strong and diffuse immunostaining (+3) in placenta of 37 weeks in IUGR group (200x).

Expression scores of caspase-3 immunostaining were 1. 0 ± 0. 4 in the preeclamptic group, 1. 2 ± 0. 4 in the IUGR group, 1. 2 ± 0. 5 in the HELLP group, and 0. 5 ± 0. 5 in the control group.

As a result, caspase-3 immunostaining score was significantly higher in each group when compared with the control group (p = 0. 002). When the authors compared each group with the control group seperately, each group stained more strongly with caspase-3 than the control group (Table 2).

Bcl-2 immunostaining scores were 1. 9 ± 0. 8 in the preeclamptic group, 2. 1 ± 1. 0 in the IUGR group, 2. 2 ± 0. 8 in the HELLP group, and 2. 3 ± 0. 5 in the control group. There was no statistically significant difference with bcl-2 immunostaining in each group when compared with the control group (p = 0. 518) (Table 2).

Discussion

Altered uteroplacental blood flow is the key event of abnormal pregnancy in either preeclampsia or IUGR [2]. A recent model for the pathogenesis of preeclampsia describes a process by which a placental factor is released into the maternal circulation causing damage to maternal endothelium with systemic inflammatory response [18].

Maternal systemic inflammatory response also occurs in normal pregnancy but is more severe in preeclampsia [19]. Placental factor seems to be the syncytial debris released into maternal circulation as a result of syncytial apoptosis, which is a part of normal cell turnover and repair [4, 10].

Apoptosis is a descriptive term for the unique morphology of cell suicide and may be a part of normal physiology or secondary to pathological conditions [20]. Apoptosis of trophoblasts has been detected in normal human placentas [4, 5] and in placentas of preeclampsia, IUGR, and HELLP syndrome [6-8].

In the literature the assessment of placental apoptosis differs due to different techniques and methods of quantification [9, 10] and as a result there is a controversy about the role of placental apoptosis in hypertensive pregnancy disorders and IUGR.

Although increased placental apoptosis in abnormal pregnancies is generally accepted [4, 5, 8, 13, 14], there are also some studies indicating reduced placental apoptosis [6, 15] in hypertensive pregnancy disorders and IUGR.

In the present study, apoptotic marker caspase-3 is significantly increased in the villous trophoblasts of patients with preeclampsia, HELLP syndrome, and IUGR compared to a control group. However placental staining of anti-apoptotic marker bcl-2 showed no statistically significant difference among groups.

In the literature, there are similar results indicating increased placental apoptosis in pregnancies complicated with IUGR and preeclampsia as in this study.

Aban *et al*. demonstrated increased placental apoptosis shown by M30 and caspase-3 staining accompanied by increased NF-κB and decreased bcl-2 expression in pregnancies complicated with IUGR and preeclampsia [13]. Ishihara *et al*. have shown increased apoptosis throughout fas antigen and bcl-2 in human term placentas complicated by either preeclampsia or IUGR [14]. Another report studying the placental bed of pregnancies complicated by preeclampsia demonstrated widespread apoptosis with a decrease in bcl-2 expression [8].

However there have also been some studies reporting decreased placental apoptosis in pregnancies complicated with IUGR and preeclampsia. Kadyrov *et al*. demonstrated decreased apoptosis in the extravillous trophoblasts in preeclamptic placentas using a M30 antibody [15] and another study by Stepan *et al*. showed that apoptotic mediators BNip3 and Nix are decreased in the villous trophoblast cells of patients with preeclampsia, HELLP syndrome and IUGR [6]. They explained the decreased apoptosis as a tolerance to chronic hypoxia in the placenta that could cause less trophoblast apoptosis as expected [6].

These apoptosis features of placenta are controversial because of methodological restrictions associated with limitations of human tissue investigations and animal studies. Basal plates of delivered placentas or curettage specimens have been used in most studies, but pathogenic events ocur in the placental bed [2]. Placental bed biopsy

specimens are available only in a few groups obtained usually at the time of cesarean section [21, 22] or from hysterectomized uteri [15, 23]. Also pathogenesis of IUGR and preeclampsia occur in early stages of human pregnancy, so it is impossible to get placental bed biopsies at that time.

In case of animal experiments, it is also difficult to create a model similar to human pregnancy because of the variations in the trophoblastic invasion among experiment animals [24, 25]. Most of the placental-related studies have focused on nuclear changes that occur in the apoptotic process which are relatively subjective criteria. However apoptosis in human trophoblasts is a very complex process consisting of many signal transduction pathways, bcl-2 regulators, caspases, and substrates [20]. Also to decrease bias in the quantification of immunohistochemical staining in the placenta, more quantitative methods such as image analysis should be preferred.

As a result, a complex cascade of apoptosis in human placenta, difficulties in getting placental bed biopsies, small numbers of study materials, and variation of trophoblastic invasion in experimental animals comprise these controversies about apoptosis of human placenta in abnormal pregnancies. These methodological problems seem to be difficult to solve at present, however for future researches we should be aware of these limitations.

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