

# Role of cited-1 and caspase-6 expression in HELLP syndrome

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**Abstract. – OBJECTIVE:** In this study, we investigated the immunohistochemical staining of cited-1 and caspase-6 expression in the placentas of pregnant women with HELLP syndrome.

**PATIENTS AND METHODS:** Placentas of 20 normotensive patients and 20 women with HELLP syndrome were processed for routine histological tissue processing. The biochemical and clinical parameters of patients were recorded. Placentas were stained with hematoxylin-eosin and cited-1 and caspase-6 immunostaining.

**RESULTS:** Placentas of normotensive patients showed normal histology. Placentas of women with HELLP syndrome showed degenerated cells, hyalinization and vacuolization. Cited-1 expression was negative in normotensive group; however, it was increased in HELLP group, especially in decidual cells, endothelial cells and other placental cells. Caspase-6 expression was negative in placental structures of normotensive groups. However, it was intense in decidual cells, vacuolar and hyalinized areas, inflammatory cells and connective tissue cells in HELLP group.

**CONCLUSIONS:** Cited-1 and caspase-6 are a marker in determining the severity of HELLP syndrome.

*Key Words:*

HELLP, Placenta, Cited-1, Caspase-6.

## Introduction

Pregnancy complication can cause maternal morbidity and mortality. Gestational hypertension (GHT), preeclampsia, eclampsia, superimposed preeclampsia, gestational diabetes mellitus (GDM), postpartum hemorrhage and infections are all common complications developed during pregnancy or in the postpartum period<sup>1,2</sup>. All pregnant patients are recommended for clinical checkup and pregnancy follow-ups. However, there is still no definitive and clear screening test to diagnose these complications. Detailed medical and obstet-

ric history is still the most commonly used method for diagnosis. Most pregnancy complications may be resolved after delivery however their long-term effect may continue in the future<sup>3,4</sup>.

Hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome was first diagnosed as severe pregnancy poisoning with hemolysis and thrombocytopenia in the records by Pritchard et al<sup>5</sup> in 1954. Another clinician called Louis Weinstein<sup>6</sup> made full definition of HELLP syndrome as intra-vascular hemolysis (H), high liver enzymes (EL) and low platelets (LP) in 1982. The incidence of HELLP syndrome in pregnancies is 0.2-0.76% in 1,000 live deliveries, the mortality rate is 0-24%, and the perinatal mortality rate is 37%. The occurrence of HELLP syndrome is highly associated with eclampsia and severe preeclampsia. Three different subgroups were identified in HELLP syndrome according to the platelet count. Clinical presentation goes similar to that of preeclampsia, hypertension or proteinuria and may not be always evident. The pathophysiology of HELLP syndrome is still not clear today. Vascular endothelial injury is the widely accepted theory. Vasoconstrictive substances are released as a result of the deterioration of vascular permeability, and it has been reported<sup>7,8</sup> that these are found at high rates in patients with HELLP syndrome.

In this study, we investigated the immune activity of cited-1 and caspase-6 proteins in placentas of women with HELLP syndrome by immunohistochemical methods.

## Patients and Methods

In our study, 20 normotensive women and 20 women with HELLP syndrome were included. Placentas were obtained from Gynecology and Obstetrics Clinics. All patients signed informed consent form. Biochemical and clinical param-

eters for each patient were recorded. Placental tissues were processed for routine paraffine wax embedding protocol.

**Histological Tissue Processing**

Placental tissues were fixed with zinc-Formalin solution (catalog No.: Z2902, Sigma-Aldrich, St. Louis, MO, US) and washed under tap water for 5 minutes. Tissues were passed through ascending alcohol series for about 24 hours. Tissues were washed with xylene 2x30 minutes and incubated within paraffin wax. 5 µm sections were cut with microtome (catalog No.: Leica RM2265, Wetzlar, Germany). Deparaffinized within xylene for 2x30 minutes, sections were brought to distilled water. Sections were stained with cited-1 and caspase-6 immunohistochemical staining<sup>9</sup>.

**Cited-1 And Caspase-6 Immunostaining**

All placental tissues were brought to distilled water. Hydrogen peroxide solution (catalog No.: TA-015-HP, ThermoFischer, Fremont, CA, USA) were dropped on sections for 20 minutes. After washing in phosphate-buffered saline (PBS) for 3x5 minutes, ultra V Block (catalog No.: TA-015-UB, ThermoFischer, Fremont, CA, USA) was applied to sections for 8 minutes. Sections were incubated with primary antibodies cited-1 and caspase-6 (AFG Scientific, USA, 1/100) at +4°C overnight. Sections were allowed to warm at room temperature for 30-60 minutes. Sections were washed with biotinylated secondary antibody (catalog No.: TP-015-BN, ThermoFischer, Fremont, CA, USA) for 14 minutes. Streptavidin-peroxidase (catalog No.: TS-015-HR, Ther-

moFischer, Fremont, CA, USA) was dropped onto sections for 15 minutes. Clearing with PBS, 3, 3' Diaminobenzidine (DAB) (catalog No.: TA-001-HCX, ThermoFischer, Fremont, CA, US) was used as chromogen. Sections were counter stained with Gill hematoxylin (catalog No.: 105174, Sigma-Aldrich, St. Louis, MO, USA). and mounted with entellan (catalog No.: 107961, Sigma-Aldrich, St. Louis, MO, USA). Slides were analyzed with Zeiss Imager A2 Zen 3.0 software (Carl-Zeiss-Straße, Oberkochen, Germany) and photomicrographed<sup>10</sup>.

**Statistical Analysis**

The data were recorded as median (minimum – maximum). Statistical analysis was done using the SPSS 25.0 software (IBM Corp., Armonk, NY, USA). Groups were binary compared with Student *t*-test. *p*<0.05 was accepted as significant level.

**Results**

**Biochemical Parameters**

Age, gravida, parity, systolic blood pressure (BP), diastolic BP, hemoglobin, platelet, glucose, urea, creatinine, alanine transaminase (ALT), aspartate aminotransferase (AST)-urine protein were recorded in women with normotensive and HELLP syndrome. Data were shown in Table I. High systolic and diastolic BP level and low platelet level were characteristics in pregnant women with HELLP syndrome. Graphical illustration of Table I was shown in Figure 1.

**Table I.** Clinical and biochemical parameters of normotensive and HELLP patients.

Parameter (N=20)	Normotensive Median (Min-Max)	HELLP Median (Min-Max)	<i>p</i> -value
Age	24 (21-35)	33 (24-39)	
Gravida	2 (0-4)	4 (1-6)	
Parity	1 (0-5)	5 (1-7)	
Systolic BP	90 (84-110)	225 (120-340)	0.028
Diastolic BP	65 (61-82)	101 (80-130)	0.015
Hemoglobin	12.5 (11-13.6)	11.5 (9.0-12.7)	
Platelet	237 (134-451)	155 (134-420)	
Glucose	75 (63-98)	88 (70-120)	
Urea	13 (11-18)	27 (17-40.00)	
Creatinine	0.54 (0.51-0.68)	0.61 (0.54-0.75)	
ALT	9 (7-19)	15 (10-70)	<0.01
AST	13 (11-35)	25 (20-73)	<0.01
2h-urine protein	111 (98-174)	1,022 (520-1,440)	<0.01

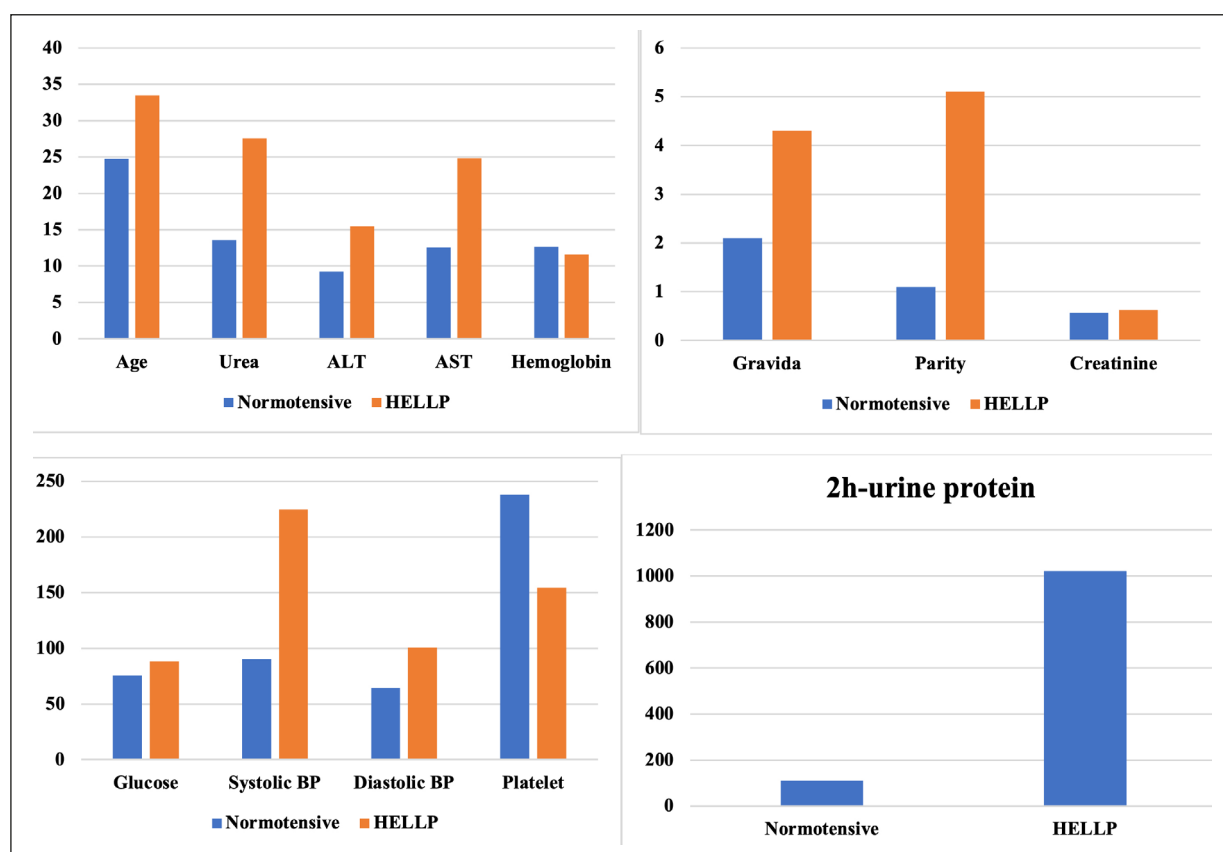


Figure 1. Graphical illustration of biochemical and demographical parameters.

### Histopathological Staining

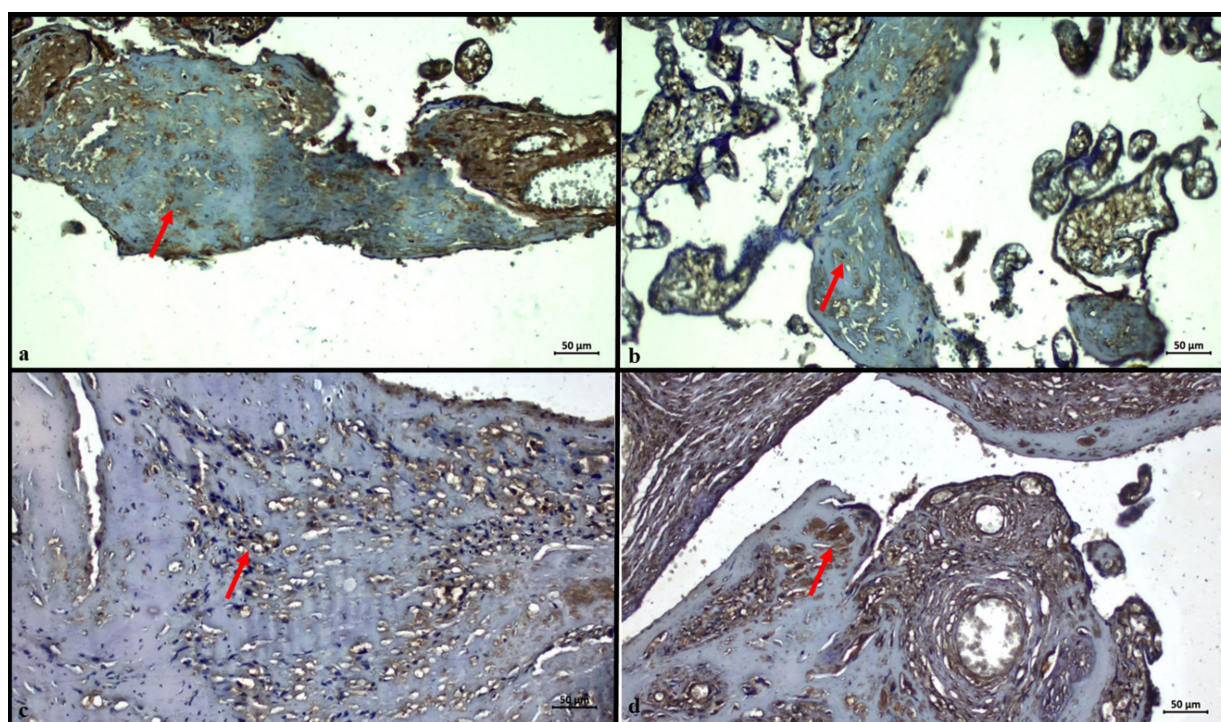
In the placentas of the control group, negative cited-1 expression was observed in decidual cells of the maternal region, syncytial areas and connective tissue areas. In some small decidual cells, cited-1 expression was positive (Figure 2a). In the placentas of patients with HELLP syndrome, hyalinization and degenerated collagen structures were observed in root villi, and apoptotic structures were observed in decidual cells. In decidual cells, degenerated cells in the hyalinized areas and vascular structures showed positive cited-1 reaction. Cited-1 expression was observed as negative in syncytial bridges and nodes (Figure 2b).

In placental sections of the control group, caspase-6 expression was negative in decidual cells of the maternal region, syncytial nodes, syncytial bridges, and connective tissue cells (Figure 2c). In the placental sections of women with HELLP syndrome, some of the decidual cells were hyperplastic and hypertrophic. Caspase-6 reaction was positive in decidual cells, vacuolar and hyalinized areas. Negative caspase-6 expression was observed in the syncytial cells of extending

root villi. Caspase-6 reaction was positive in aggregated inflammatory cells, connective tissue cells and floating villi (Figure 2d).

### Discussion

Pregnancy complications can cause abnormal placental development. Common complications can be listed as preeclampsia, HELLP, gestational diabetes mellitus (GDM), placenta previa and accreta. HELLP syndrome may have short- and long-term effects during pregnancy and postpartum. HELLP is also known to affect liver, kidney, and other organs. Vinnars et al<sup>11</sup> studied 196 women diagnosed with HELLP syndrome. In the histopathology of placenta, intervillous thrombosis, abruption, infarction were more common in women with HELLP syndrome. HELLP syndrome can also cause renal dysfunction due to glomerular endotheliosis and liver pathology due to apoptosis of liver sinusoidal endothelial cells, causing lesion in renal and hepatic histopathology<sup>12</sup>. Weiner et al<sup>13</sup> studied preeclampsia and



**Figure 2.** Normotensive and HELLP syndrome placentas with *cited-1* (a-b) and caspase6 (c-d) immune staining. Decidual cells (arrows). Scale Bar: 50  $\mu$ m, magnification: 20X.

HELLP syndrome placentas histopathologically. They found that both complications showed similar pathology, but in the placentas of the HELLP group, vascular and villous lesions and maternal malperfusion were elevated.

*Cited* is a coactivator in transcription and possibly responsible for melanocytes pigmentation. It mediates events in transcription regulated by estrogen<sup>14</sup>. *Cited* consists of four nuclear proteins as *cited-1*, *cited-2*, *cited-3* and *cited-4*. Since the *cited* protein has no DNA binding site, its role is mainly transcriptional regulator<sup>15</sup>. Sriraman et al<sup>16</sup> studied progesterone receptor in cultured granulosa cells and found that progesterone receptor induced many genes that regulated granulosa cells activity. One of the genes was *cited-1* that is affected by progesterone receptor during ovulation. Hatzirodos et al<sup>17</sup> investigated the transcriptome profile of granulosa cells in bovine ovarian follicles. The authors found that as follicle develops larger, transcriptional regulators were high in number. One of the regulators was *cited-1*. In our study, control group showed mainly negative *cited-1* expression in placental structures, decidual cells and connective tissue cells (Figure 2a). In HELLP group, decidual cells and connective fibers were degenerated. *Cited-1* expression was

increased in decidual cells, endothelial cells and other placental cells (Figure 2b).

Caspases are cysteine proteases that are involved in cell death and in immune responses. caspase-6 is an executioner caspase. Its role in apoptosis is well known however other roles remain unclear. The placenta's development depends on the implantation in the uterus and invasion of the decidual plate by trophoblast cells<sup>18,19</sup>. Cali et al<sup>20</sup> studied immunoexpression of caspase-3 in placentas of HELLP complicated pregnancies in 5 women. They recorded that caspase-3 immune score was significantly higher than control patients and increased in the villous trophoblasts. The authors stated that caspase-3 could be used as a marker for HELLP syndrome immunohistochemically. John et al<sup>21</sup> investigated the role of apoptosis and cell death in placental trophoblastic cells of patients with HELLP syndrome. They found that the level of cell death and caspase activation were increased in placentas of women with HELLP syndrome when compared to normotensive patients. The authors<sup>21</sup> stated that apoptotic signal is increased in HELLP syndrome. Control group showed mainly negative caspase-6 expression decidual cells and placental structures such as syncytial nodes, syncytial bridges, and connective tissue cells (Figure

2c). In HELLP group, decidual cells were degenerated. Caspase-6 expression was increased in decidual cells, vacuolar and hyalinized areas, inflammatory cells and connective tissue cells (Figure 2d).

## Conclusions

HELLP syndrome caused the degeneration of syncytial and decidual cells. Cell proliferation precursor cited-1 and executioner caspase-6 are important signaling molecules in determining inflammation and cell apoptosis and may be markers in determining the severity of HELLP syndrome.

### Ethics Approval

Ethical approval was taken from Dicle University Non-Interventional Clinical Research Ethical Committee (2023/33).

### Informed Consent

All patients were informed about the study. All patients signed and approved the informed consent.

### Availability of Data and Materials

All generated materials and data were presented in the study.

### Authors' Contributions

EÖ, SAA and FA read and approved final version of the manuscript. All authors equally contributed to the manuscript in writing, editing, drafting, conceptualization stage.

### Conflict of Interest

The authors declare that they have no conflict of interests.

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