Expression of E-cadherin, N-cadherin, and Cytokeratin 18 and 19 in Placentas of Women with Severe Preeclampsia

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Abstract

BACKGROUND: Although the exact mechanism leading to preeclampsia is not fully understood, abnormal trophoblast invasion contributes to its pathogenesis. Keratins and cadherins are known to play roles in the regulation of trophoblast proliferation. However, studies describing the association between keratins, cadherins, and preeclampsia are limited.

AIM: The current study was conducted to investigate the association of these proteins with severe preeclampsia in Sudanese women.

METHODS: A case–control study was conducted at Madani Maternity Hospital, Sudan. The cases included women with severe preeclampsia (n = 56) and healthy pregnant women as controls (n = 56). The assessment of keratin and cadherin was performed using immunohistochemical staining.

RESULTS: There was no significant difference between the two groups in their mean age or parity. We found no significant differences in the expression of the markers E-cadherin, N-cadherin, or cytokeratin 18 and 19 in the placentas from individuals with preeclampsia versus controls. The number of placentas with severe preeclampsia versus controls expressing the E-cadherin, N-cadherin, cytokeratin 18, and cytokeratin 19 markers was 46 (82.1%) versus 48 (85.7%) (p = 0.121), 4 (7.1%) versus 0 (0%) (p = 0.126), and 11 (19.6%) versus 11 (19.6%) (p = 0.532), respectively. There was also no significant difference in the intensity of staining of these four markers (E-cadherin, N-cadherin, and cytokeratin 18 and 19) between severe preeclampsia and control placentas.

CONCLUSION: Together, these results indicate that in this setting, the expression of E-cadherin, N-cadherin, CK18, and CK19 is not associated with severe preeclampsia.

Introduction

Preeclampsia is defined as the occurrence of hypertension and proteinuria in the second half of pregnancy (i.e., after 20 weeks of gestation) in women who had no previous hypertension or proteinuria. It is one of the most common medical disorders in pregnancy worldwide, with a prevalence of around 3–8% [1], [2]. It is a serious pregnancy-related complication that can lead to adverse effects for both the mother and the fetus [1], [3]. Whereas most cases of preeclampsia are mild and symptomless, it may also occur in a severe form, such as hemolysis, elevated liver enzymes, low platelets-syndrome, or may lead to cerebral manifestations and eclampsia [4]. Although the exact pathophysiology and pathogenesis of preeclampsia is not completely understood, it is theorized that poor/abnormal placentaentation during early pregnancy can lead to placental ischemia and release of vasoactive substances, with consequent endothelial activation and dysfunction [5].

The proliferation and differentiation processes of the human chorionic villous and extra villous trophoblast are associated with alterations in the expression of keratins and cadherins at various stages of pregnancy [6], [7], [8]. Keratins are glycoproteins that are expressed by a variety of tissues. Cadherins mediate adhesion and play important roles in the formation of tissues during gastrulation [9]. Keratins are a family of intermediate filament-forming proteins with specific physiochemical properties that contribute to the formation of the cytoskeleton; cytokeratin 18 and 19 are specifically expressed by epithelial cells, such as placental trophoblasts [10]. Cadherins mediate homophilic cell-cell adhesion and cell-cell recognition [11]. There is a limited amount of research regarding the association between E-cadherin, N-cadherin, keratin 18, keratin 19, and preeclampsia [12], [13], [14], [15], [16]. The current study was conducted to investigate the association of these proteins with severe preeclampsia in Sudanese women.
Methods

A case–control study was conducted at Madani Maternity Hospital, Sudan, during the period between September 2017 and December of 2017. Madani hospital is a tertiary hospital caring for referred women. The cases examined were women with severe preeclampsia. Preeclampsia is defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg on two occasions at least 4 h apart after 20 weeks of gestation in a previously normotensive patient and proteinuria ≥0.3 g in a 24-h urine specimen. Severe preeclampsia is defined as the occurrence of diastolic blood pressure ≥110. Health pregnant women, similar in age and similar in other characteristics were used as controls. The control women did not have blood pressure values >139/89 mmHg or proteinuria. Women pregnant with twins, or with previous histories of hypertension, renal disease, diabetes, or liver disease were excluded from the study. After signing an informed consent, both the obstetrics and medical history (age, parity, and gestational age) were recorded from each woman (cases and controls) using a questionnaire that was completed and filed by a trained medical officer. Maternal weight and height were measured and body mass index was calculated and expressed as weight (kg)/height (m)^2. The Institutional Ethics Committee approved the study protocol, and informed consent was obtained from each subject.

Immunohistochemistry

Immediately after delivery, placental samples were fixed in 10% neutral buffered formalin solution. Samples were cut into to 3–5 mm-thick tissue slices and processed in a standard manner for the preparation of 4 µ-thick paraffin sections. Sections were de-waxed with xylene and hydrated through descending alcohol (100%, 90%, 70%, and 50% ethanol and distilled water). The sections then were treated for antigens, which were retrieved by pre-heated ethylenediaminetetraacetic acid pH 9 (Boster biological Technology Co. Ltd., China) in a microwaveable chamber, microwaved (at 98°C) for 20 min. Endogenous peroxidase was blocked using a microwaveable chamber, microwaved (at 98°C) for 20 min. Endogenous peroxidase was blocked using 0.03% hydrogen peroxide for 5 min. Tissue sections were incubated with mouse primary antibody in a humid chamber for 45 min using dilutions as follows: (K19 clone number RCK108, code: BM3267, 1:50; K18 clone number DC10, code: AM0094, 1:50; E-cadherin clone number ECH-6, code: AM0104, 1:50; N-cadherin clone number 6G11, code: AM0170, 1:50) (Gene tech company limited, Shanghai, China), and subsequently washed with phosphate buffer saline at pH 7.4 (SIGMA, USA). Next, sections were incubated with horse-radish peroxidase labeled polymers mouse antibody for 30 min (code: GK500705, Gene tech company limited, Shanghai, China). A brown color was developed by adding diaminobenzidine tetra hydrochloride (Gene tech company limited, Shanghai, China) for 10 min. The slides were lightly counterstained using Mayer hematoxylin negative control, prepared from the same tissues block, but incubated in the phosphate buffer solution instead of the primary antibody. Positive control sections were added to the process with the placental tissue sections in the same run, the staining intensity of E-cadherin, CK18 and CK19 in two sites of placenta was tested as an internal control and no difference in expression was found. Twenty fields were examined for each section by an expert pathologist blinded to the tissue identity and scored for intensity and location of staining as follows: Level of staining assessed as 0 = no staining, 1 = low staining, 2 = medium staining, and 3 = intense staining. All sections were stained in the same batch to control for inter-batch variation.

Data analysis

Data were entered into the computer using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) for Windows. Continuous and categorized data were compared between the two groups (severe preeclampsia and healthy controls) using Student’s t-test and Chi-square test, respectively. p < 0.05 was considered significant.

Results

Fifty-six placentas were investigated in each arm of the study. There were no significant differences in the mean (SD) age, parity, or hemoglobin levels between the two groups. Compared to controls, women with severe preeclampsia had significantly lower gestational age and birth weights (Table 1).

| Variables            | Severe preeclampsia (n=56) | Controls (n=56) | p-value |
|----------------------|-----------------------------|-----------------|---------|
| Age, years           | 28.9 (6.2)                  | 28.5 (4.9)      | 0.7310  |
| Parity               | 3.1 (1.9)                   | 2.8 (1.6)       | 0.902   |
| Gestational age, weeks | 36.2 (2.9)       | 37.8 (0.9)     | <0.001  |
| Body mass index, kg/m² | 24.6 (2.1)      | 24.4 (2.3)     | 0.677   |
| Hemoglobin, g/dl     | 10.2 (1.3)                  | 9.9 (1.8)       | 0.284   |
| Birth weight, g      | 2560 (451)                 | 3019 (321)      | <0.001  |

There were no significant differences in the absolute expression of these markers. Of the placentas tested from severe preeclampsia and control patients, there were 46 (82.1%) versus 46 (82.1%) (p = 0.988), 54 (96.4%) versus 48 (85.7%) (p = 0.121), 4 (7.1%) versus 0 (0%) (p = 0.126), and 11 (19.6%) versus 11 (19.6%) (p = 0.532) that expressed E-cadherin, N-cadherin, and cytokeratin 18 and 19, respectively.

There was no significant difference in the intensity of staining of the four markers (E-cadherin, N-cadherin, and cytokeratin 18 and 19) between the placentas of severe preeclampsia and controls (Table 2). Only one placenta from a severe preeclampsia patient...
expressed the four markers (E-cadherin, N-cadherin, and cytokeratin 18 and 19), \( p = 0.500 \). Both E-cadherin and N-cadherin were expressed in 43 (76.8\%) versus 40 (71.4\%), \( p = 0.333 \) of the placentas of the severe preeclampsia and controls, respectively. Only 2 (3.2\%) versus 0 (0\%), \( p = 0.248 \) of placentas of severe preeclampsia cases expressed cytokeratin 18 and 19 (Figure 1).

**Discussion**

The main findings of the current study indicate that there were no significant differences in the expression of keratin 19, keratin 18, and N-cadherin in placentas from women with severe preeclampsia, compared to placentas from healthy controls. Interestingly, Li et al. reported no significant differences in the expression of N-cadherin between preeclampsia and control cases. However, they did report a significant increase in the expression of E-cadherin, cytokeratin 18, and cytokeratin 19 in preeclampsia placentas, compared to normotensive pregnancies [12]. Moreover, Du et al. reported that the expression of E-cadherin is higher, whereas the expression of N-cadherin is lower in the placentas of preeclamptic women, compared to the placentas of the healthy controls [17].

A significant increase in the expression and significantly higher level of cytokeratin 18 has recently been recently in placental samples from women with preeclampsia compared to controls [13]. Likewise, an upregulation of E-cadherin expression has been reported in preeclampsia patients [16]. Tempfe et al. reported a significant association between cytokeratin
19 and preeclampsia, especially the severe form of preeclampsia [14]. On the other hand, keratins 18 and 19 have been reported to be downregulated in villous trophoblasts in preeclampsia [15].

It is worth mentioning that E-cadherin and cytokeratin 18 have been proposed to be markers for apoptosis, which is a feature of preeclampsia that contributes to placental malfunctioning and impairs maternal-fetal transfer functions [18]. Al-Nasiry et al. reported that trophoblast fusion is associated with a downregulation of E-cadherin and defective placentation in preeclampsia [19].

The attachment of cells to each other is mediated by interactions between homophilic cadherin molecules that include Epithelial (E) E-cadherin and Neural (N) N-cadherin; these cadherin molecules are linked to different cytoskeletal elements, for example, keratins 19 and 18 [20]. N-cadherin is associated with the epithelial–mesenchymal transition and is imperative for well-coordinated and coherently layered villous cytrophoblasts, which change into extravillous, migratory and invasive trophoblasts [8]. E-cadherin is essential for embryonic development as it is expressed in epithelial cells and the localization of cadherins is highly relevant to the morphology of trophoblasts at different stages of placental development and villous differentiation [21].

### Conclusion

Caution should be taken when comparing these findings with later studies because, although we found that the correlations were not statistically significant, there were clear differences within the set of affected women, and the sensitivity of the detection methods, and the sampling area in the placenta, is very important for determining the expression of such markers.

### Limitations of the study

The assessments of keratin and cadherin were performed only through immunohistochemical staining.

### Declarations

#### Ethics

The study was approved by the ethics committee at Omdurman Islamic University, Khartoum, Sudan. Written informed consent from patients used in this study was obtained to safeguard the rights and autonomy of the patient.

### Availability of Data and Materials

Data are available from the corresponding author on reasonable request.

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### Authors’ Contributions

Authors SEO and IA were responsible for the overall study. EME designed and conducted the clinical portions. SEO, MMS, and AAM conducted the laboratory work. EME and IA conducted the statistical analyses. SEO and AAM helped to draft and revise the manuscript. All authors read and approved the final manuscript.

### References

1. Lo JO, Mission JF, Caughey AB. Hypertensive disease of pregnancy and maternal mortality. Curr Opin Obstet Gynecol. 2013;25(2):124-32. PMid:23403779
2. Abalos E, Cuesta C, Carroli G, Qureshi Z, Widmer M, Vogel JP, et al. Pre-eclampsia, eclampsia and adverse maternal and perinatal outcomes: A secondary analysis of the World
observed in preeclampsia were not correlated with disease severity. Placenta. 2014;35(8):625-31. https://doi.org/10.1016/j.placenta.2014.04.010
PMid:24857367

13. Hefler LA, Tempfer CB, Bancher-Todesca D, Schatten C, Husslein P, Heinze G, et al. Placental expression and serum levels of cytokeratin-18 are increased in women with preeclampsia. J Soc Gynecol Investig. 2001;8(3):169-73. https://doi.org/10.1177/107155760100800308
PMid:11390252

14. TempferCB, Bancher-TodescaD, ZeislerH, SchattenC, HussleinP, Gregg AR. Placental expression and serum concentrations of cytokeratin 19 in preeclampsia. Obstet Gynecol. 2000;95(5):677-82. https://doi.org/10.1097/00006250-20000500-00009
PMid:10775228

15. Ahenkorah J, Hottor B, Byrne S, Bosio P, Ockelford CD. Immunofluorescence confocal laser scanning microscopy and immuno-electron microscopic identification of keratins in human maternal-fetal interaction zone. J Cell Mol Med. 2009;13(4):735-48. https://doi.org/10.1111/j.1582-4934.2008.00363.x
PMid:18466353

16. Li HW, Cheung AN, Tsao SW, Cheung AL, O WS. Expression of e-cadherin and beta-catenin in trophoblastic tissue in normal and pathological pregnancies. Int J Gynaecol Pathol. 2003;22(1):63-70. https://doi.org/10.1097/00004347-200301000-00013
PMid:12496700

17. Du L, Kuang L, He F, Tang W, Sun W, Chen D. Mesenchymo-to-epithelial transition in the placental tissues of patients with preeclampsia. Hypertens Res. 2017;40(1):67-72. https://doi.org/10.1038/hr.2016.97
PMid:27511055

18. Longtine MS, Chen B, Odibo AO, Zhong Y, Nelson DM. Villous trophoblast apoptosis is elevated and restricted to cytotrophoblasts in pregnancies complicated by preeclampsia, IUGR, or preeclampsia with IUGR. Placenta. 2012;33(5):352-9. https://doi.org/10.1016/j.placenta.2012.01.017
PMid:22341340

19. Al-Nasiry S, Vercruysse L, Hanssens M, Luyten C, Pijnenborg R. Intertitial trophoblastic cell fusion and E-cadherin immunostaining in the placental bed of normal and hypertensive pregnancies. Placenta. 2009;30(6):719-25. https://doi.org/10.1016/j.placenta.2009.05.006
PMid:19616845

20. Garrod D, Chidgey M. Desmosome structure, composition and function. Biochim Biophys Acta. 2008;1778(3):572-87. PMid:17854763

21. Babawale MO, Van Noorden S, Pignatelli M, Stamp GW, Elder MG, Sullivan MH. Morphological interactions of human first trimester placental villi co-cultured with decidual explants. Hum Reprod. 1996;11(2):444-50. https://doi.org/10.1093/humrep/11.2.444
PMid:8671240

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