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Angiotensin-1 and vimentin expression and ultrastructural examination in severe preeclampsia complicated by HELLP syndrome changes in the structure of the umbilical cord

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Abstract. – OBJECTIVE: The purpose of this study was to examine the histopathologic, ultrastructural and immunohistochemical changes in the umbilical cord in women diagnosed with HELLP syndrome.

MATERIALS AND METHODS: Postpartum umbilical cords of 40 patients at the 35-38th week of pregnancy were included. 20 severe preeclamptic (HELLP) and 20 normal umbilical cords were used. After the follow-up of tissue parts of 10% formaldehyde solution for histopathology and immunohistochemistry, histopathological and angiopoietin-1 and vimentin antibodies were examined as immunohistochemical after routine paraffin follow-up. For electron microscope analysis, umbilical cord samples were taken into 2.5% glutaral aldehyde solution.

RESULTS: In the statistical comparison, mean difference in increased diameter and additional anomaly on the ultrasound of preeclamptic patients was statistically different compared to control patients. In the HELLP group, hyperplasia and degenerative changes, pyknosis of the endothelial cell nuclei of the vessels and apoptotic changes in some regions were observed. Immunohistochemical analysis showed that endothelial cells, basal membrane and fibroblast cells in the HELLP group expressed high levels of vimentin. Angiotensin-1 expression was increased in amniotic epithelial cells, endothelial cells and some pericyte cells.

CONCLUSIONS: As a result, it was observed that the signaling that started with trophoblastic invasion with the effect of hypoxia in severe preeclampsia and continued with dysfunction in endothelial cells was parallel to the increase in angiotensin and vimentin receptors. It is thought that the ultrastructural change in endothelial cells may cause disruption of the collagenized structure in Wharton gel, which supports this, and may cause adverse effects in fetal development and nutrition.

Key Words:
HELLP syndrome, Angiotensin-1, Vimentin, Umbilical cord.

Introduction

It is known that preeclampsia (PE) is generally characterized by high blood pressure and proteinuria, and especially when the diastolic blood pressure reaches or exceeds 110 mmHg, the severity of the preeclampsia picture may increase and it becomes more fatal[1]. When we look at the studies[2], it is seen that almost 10% of women have high blood pressure during pregnancy, and it is generally seen that this situation complicates 2% to 8% of pregnancies with PE. In addition, studies[3,4] have shown that it triggers an immune response against maternal endothelial dysfunction, which induces thrombotic microangiopathy with endothelial damage and thrombocyte-fibrin thrombus in micro vessels, before complications such as hypertension, proteinuria and edema, which are generally manifested clinically. However, HELLP syndrome, which is one of the most important features of severe preeclampsia, has generally been shown[5] to be a very serious danger. Structural abnormal changes in the umbilical cord as a result of HELLP have been demonstrated on the verge of studies[6,7] that significantly affect fetal development. In addition, in severe preeclampsia (HELLP), hemolysis causes high liver enzymes and low platelet count. As a result, it is revealed that an increase in severe endothelial damage is observed[8,9]. One of the most important properties of Angiopoietin-1 (Ang1) is endothelial permeability, which occurs as a result

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of the inhibition of leukocyte-endothelial interactions and plays an important role as a critical factor for vascular stabilization, permeability and endothelial survival[10]. In addition, it is revealed in studies[11] that it will release the vascular endothelial growth factor (VEGF) by genetically changing the root cells obtained from the adipose tissue. However, a type III intermediate filament cytoskeleton protein expressed from stem cells of mesenchymal origin is termed vimentin. Studies[12] showed that vimentin is mainly found in fibroblasts, endothelial cells and cells of the immune system. At the cellular level, the importance of vimentin has many important biological roles. Other studies[13,14] showed that vimentin is involved in cell proliferation, cell adhesion, cell migration, differentiation and aging processes. In addition to these important features, it provides an epithelial-mesenchymal transition (EMT) and has an important role in cell migration, although it causes the opening of epithelial barriers[15,16]. In this study, we aimed to examine the histopathological, ultrastructural and immunohistochemical changes in the umbilical cord of women diagnosed with HELLP syndrome.

Materials and Methods

Ethical approval of the study was obtained from Van Akdamar Hospital Non-Interventional Clinical Trials Ethics Committee. Obtained umbilical cord samples were obtained from Van Akdamar Hospital (Gynecology and Obstetrics Department Clinic). The umbilical cords of the babies who were born in the 2nd week were removed. By creating 2 groups, a total of 40 units were obtained as the group with HELLP syndrome (n=20) and the group with normal umbilical cord (n=20). New onset of hypertension (systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg) and proteinuria (>300 mg/24 hours) were observed in all patients included in the HELLP syndrome group. Generally, it is revealed that the definition of HELLP syndrome occurs when three of the important criteria are positive in the absence of other pathological conditions: lactate dehydrogenase (LDH) >600 U/L, aspartate aminotransferase (AST) ≥ 70 U/L or alanine aminotransferase (ALT) ≥ 70 U/L, platelet count <100,000 cells/mm[11].

In this study, we provided rapid clamping of each umbilical cord at birth. The umbilical cord, 8-10 cm long, was then cut and removed. These cross-section samples obtained were placed in 10% formaldehyde buffered liquid. Afterwards, these sections were dehydrated in the ethanol series, cleaned in xylene and embedded in paraffin. As a result of these sections, 4 µm sections were cut and turned into slides. These were stained with hematoxylin-eosin.

Immunohistochemical Examination

Protocol was done according to Durgun et al[17]. The obtained umbilical cord sections were washed with distilled water to perform immunohistochemistry. Then, for the antigen application process, the application was carried out in a 700 W microwave oven for 10 minutes in citrate buffer solution with pH: 6.0. After this process, the obtained sections were allowed to cool for up to 25 minutes at room temperature and washed in distilled water for 2x4 minutes. As a result of these procedures, 3% hydrogen peroxide (H_2O_2) was used for 10 minutes in order to apply endogenous peroxidase blockade. The tissues we obtained were both washed with distilled water and with phosphate buffered saline (PBS). After performing the washing procedures, the sections obtained were incubated overnight at +4°C with mouse monoclonal anti-angiotensin-1 antibody (1:100) and mouse monoclonal vimentin antibody (1:100). The sections that were kept for the next day were cleaned with PBS and applied with a secondary antibody solution for 20 minutes. After PBS treatment, streptavidin peroxidase solution (Streptavidin Peroxidase, Lab Vision, Fremont, CA, USA) was applied for 15 minutes. After the obtained sections were washed 3 times in PBS, DAB chromogen solution was applied for 8 minutes. Afterwards, the sections were washed with distilled water and counterstained with Harris hematoxylin for 2 minutes. The obtained sections were then allowed to be viewed under a light microscope with the imager A2 Zeiss.

Electron Microscopic Technique

The dissection of the umbilical cords was carried out in a 2.5% glutaraldehyde (pH: 7.2) solution with pH: 7.4 0.1 M phosphate buffer. Initial fixation of the tissues was performed for 24 hours in 2.5% glutaraldehyde. Then it was washed with pH: 7.4 0.1M phosphate buffer by changing it 3 times every 15 minutes. The second fixation was done in Osmium tetroxide by rotating the rotator for 2 hours at room temperature. The tissue was washed 3 times and 15 times in phosphate buffer. In the dehydration process, 50%, 70%, 90% ethyl alcohol at +4 ºC 2 times for 15 minutes; 96% and 100% ethyl alcohol were also applied 2 times for 30 minutes.

Afterwards, the tissues were kept in propylene oxide twice for 30 minutes and a 1:1 mixture of propylene oxide and araldite in the rotator for 2 hours. After the tissues were kept in pure araldite in the rotator for one night, embedding was done. The polymerization process was carried out in an oven at 60°C for 2 days. Thin sections of 70-100 nm were taken on 200 mesh copper grids and stained in 2% uranyl acetate for 1 hour. After washing the grids with Phosphate buffer, they were treated with lead acetate for 15 minutes. After treatment with lead acetate, they were washed again so that the samples were ready for the examination under the electron microscope. Sections were examined with Jeol Brand JEM-1010 model transmission electron microscope in Dicle University Science and Technology Application and Research Center and cytopathological changes were photographed with GATAN brand, 782 side entry ES500W Erlangshen Model CCD camera connected to the same electron microscope.

Statistical Analysis

In order to perform the statistical analysis of the obtained data, SPSS Statistics software version 24 (IBM Corp., Armonk, NY, USA) was used and evaluated. The obtained data were analyzed by applying normality tests. In order to make a better evaluation of the obtained data, the Mann-Whitney U test was used to make a pairwise comparison in the SPSS program. A *p*-value lower than 0.05 was accepted as statistically significant at the threshold of evaluation and results obtained.

Results

Statistical analysis of maternal age, gestational age (GA), diameter at diagnosis, diameter increased, thrombosis, turbulent flow, additional anomaly on ultrasound, fetal intraabdominal vein varix (FIUVV)-indicated delivery, GA at delivery, small-for-gestational age, intrauterine fetal demise parameters were listed in Table I. The values of preeclampsia group were compared to control group. Mean difference in increased diameter and additional anomaly on ultrasound of preeclamptic patients was statistically different comparing to control patients.

Histological staining, immunohistochemical staining and electron microscopy examination was shown in Figure 1.

Table I. Statistical analysis of maternal and fetal parameters for control and preeclamptic patients.

Parameter	Groups	n	Mean±SD	($p<0.05$)
Maternal age (years)	(1) Control	20	29.00±4.81	
	(2) Preeclampsia	20	27.00±5.20	
GA at diagnosis (weeks)	(1) Control	20	29.00±4.62	
	(2) Preeclampsia	20	29.08±5.33	
Diameter at diagnosis (mm)	(1) Control	20	13.12±4.85	
	(2) Preeclampsia	20	13.00±3.45	
Diameter increased	(1) Control	20	13.80±3.78	(2)
	(2) Preeclampsia	20	15.40±2.21	(1)
Thrombosis	(1) Control	20	0.00±0.00	
	(2) Preeclampsia	20	0.00±0.00	
Turbulent flow	(1) Control	20	7.90±1.49	
	(2) Preeclampsia	20	7.70±1.30	
Additional anomaly on ultrasound	(1) Control	20	15.40±8.94	(2)
	(2) Preeclampsia	20	13.80±3.29	(1)
FIUVV-indicated delivery	(1) Control	20	23.10±7.29	
	(2) Preeclampsia	20	23.80±8.52	
GA at delivery (weeks)	(1) Control	20	39.00±12.73	
	(2) Preeclampsia	20	39.05±14.96	
Small-for-gestational age	(1) Control	20	15.40±4.98	
	(2) Preeclampsia	20	15.60±4.20	
Intrauterine fetal demise	(1) Control	20	7.70±2.95	
	(2) Preeclampsia	20	7.90±2.78	

GA: gestational age; FIUVV: Fetal intraabdominal vein varix.

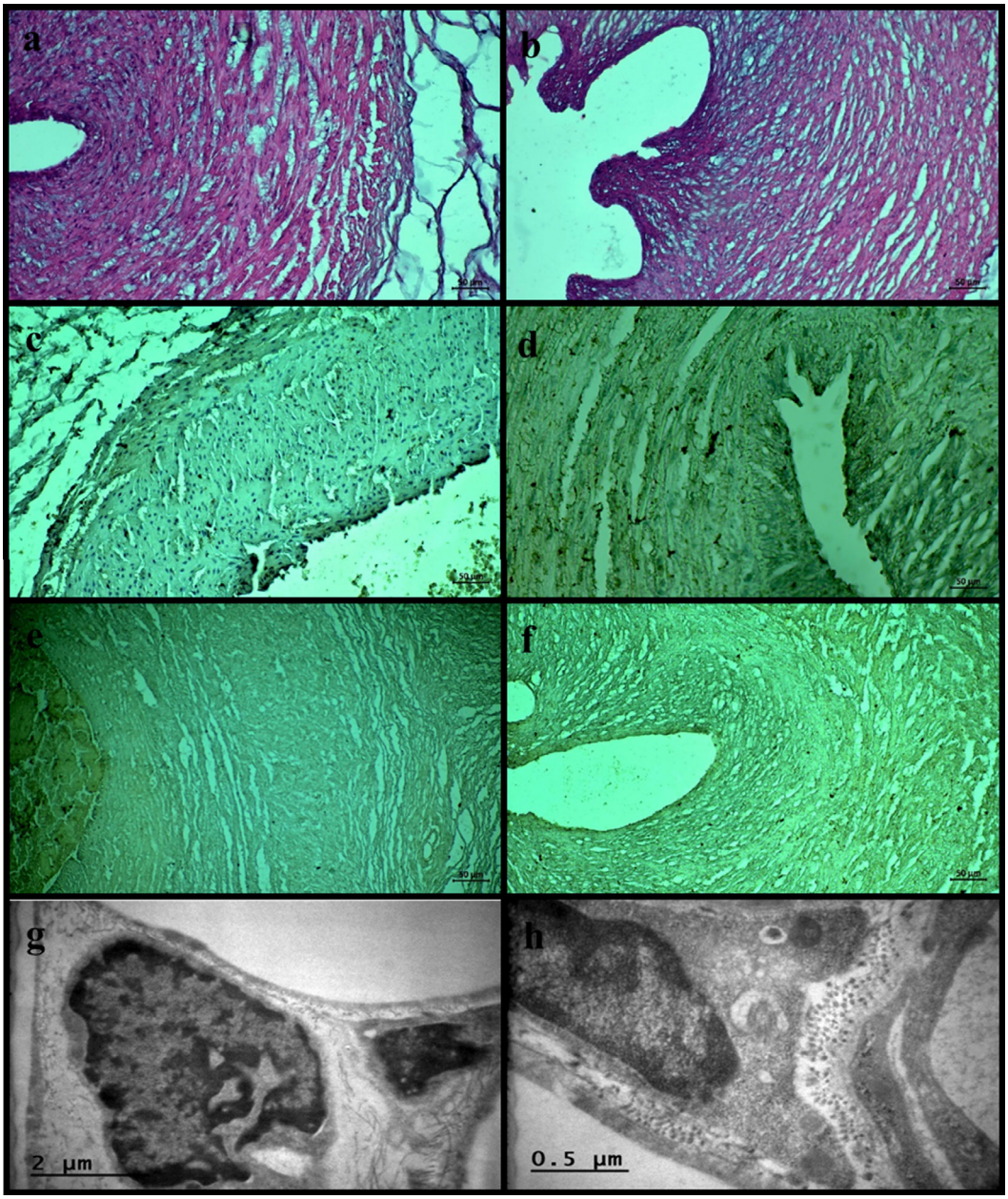

Figure 1. Hematoxylin-Eosin staining: (**a**), In the control group, the amniotic epithelium was regular and flat, and the basement membrane structure was normal. In the umbilical cord blood vessel lumens, endothelial cells appeared flat and slightly protruding into the lumen. Collagen fibers were parallel to each other in Wharton gel, solitary fusiform fibroblast cells and some mesenchymal cells were seen between them. **b**, In the HELLP group sections, hyperplasic and degenerative amniotic epithelial cells, pyknotic endothelial cell nuclei, and apoptotic changes were observed muscle layer. In the Wharton gel region, an increase in the extracellular matrix and hyperplasia in fibroblast cells were observed with the examination of collagen fibers. Scale Bar: 50 µm, magnification: 20X.
Angiotensin-1 immunostaining: (**c**), In the control group, angiotensin-1 expression was weakly observed in the amniotic epithelium outside the umbilical cord, in blood vessels endothelial cells, fibroblast and pericyte cells. **d**, It was observed that the expression of Angiotensin-1 increased in the amnion epithelial cells, endothelial cells and some pericyte cells in the HELLP group, and angiogenetic and inflammatory reaction characteristics were induced. Vimentin-1 immunostaining: (**e**), In the control group, vimentin reaction was weak in the basement membrane of the amniotic epithelium, in the intimal layer of the blood vessel, and in the smooth muscle cells. **f**, In the HELLP group umbilical cord section, an increase in vimentin expression was observed in the basal membrane of the amniotic epithelium and in the fibroblast cells of the Wharton's jelly, in the blood vessels, in the smooth muscle cells in the tunica media layer. Scale Bar: 50 µm, magnification: 20X.
Ultrastructural examination: (**g**), In control group, intensity of the chromatin was low, blood cells were protruding towards the lumen. In addition, mitochondrial structures were regular. Scale Bar: 2 µm. **h**, In the HELLP group, a significant hyperplasia in endothelial cells, degenerative changes in mitochondria, degeneration and thinning of collogen structures in the intimal area were observed. Scale Bar: 0.5 µm.

Discussion

Systemic vasoconstriction and hypertension as a result of a damage to the endothelial region of the vessels participating in the maternal circulation were caused by hypoxia complication. Among the complications that occur, hemolysis and HELLP syndrome cause an elevation in liver enzymes and thrombocytopenia, as well as systemic capillary endothelial damage[18]. The main cause of maternal clinical symptoms in preeclampsia is endothelial dysfunction. Endothelium-dependent vascular volume control and vasoconstriction cause hypertension. Increased capillary permeability causes fluid loss into the third space, hemoconcentration, and edema; increased glomerular permeability leads to proteinuria and a negative picture develops with coagulation mechanism in extensive intravascular coagulation. The reduction in capillary sprouting capacity, as a result of reduced microvascular density, is the main determinant of increased vascular resistance, leading to the progression of hypertension[19,20]. Mechanisms that associate high blood pressure with capillary dysfunction include endothelium-induced oxidative stress[21], increased anti-angiogenic activation[22], and impaired proliferative and regenerative capacity. The umbilical cord is lined with an epithelium derived from the surrounding amnion. The source of glycoprotein microfibrils and collagen fibrils in Wharton's jelly is fibroblast cells[23]. Wharton gel is a thin barrier that acts as a protective membrane in the wall structure of veins and arteries. In a study[24] on placenta previa, it was reported that the accumulation of collagen fibers in Wharton's jelly causes deterioration in the structure of blood vessels and causes a significant enlargement. Authors also stated that there is a decrease in the diameter of the umbilical cord artery wall in patients with preeclampsia. It has been stated[25] that maternal vascular endothelial dysfunction resulting in impaired synthesis of vasodilators or excessive production of vasoconstrictors in severe preeclampsia, as well as increased sensitivity of the vascular system to endogenous suppressive substances, can significantly affect tissue damage. Balsak et al[5] have stated that systolic and diastolic blood pressure, liver function tests and LDH, platelet count tests and LDH, platelet count in HELLP are significantly lower in HELLP (Table I).

In patients with HELLP syndrome, the number of white blood cells (WBC) has increased significantly and blood vessel diameters and amnion membrane thickness were significantly different according to the control group. In a study[26], the histopathological examination of the HELLP group was observed to thicken the degeneration and basal membrane in the endothelial cell on the blood vessel wall. There were also degeneration and edema areas in hyperplasia and collagenous fibers in the muscle cell. In addition to similar results in our study, the authors stated that apoptotic changes in some areas of pyknosis in endothelial cell and in collagenous fibers in the Wharton gel region. In the control group, the amniotic epithelium was regular and flat, and the basement membrane structure was normal. In the umbilical cord blood vessel lumens, endothelial cells appeared flat and slightly protruding into the lumen. Collagen fibers were parallel to each other in Wharton gel, solitary fusiform fibroblast cells and some mesenchymal cells were seen between them (Figure 1a). In HELLP group, umbilical cord sections showed hyperplasia and degenerative changes in some of the amniotic epithelial cells, pycnotic endothelial cell. In the Wharton gel region, an increase in the extracellular matrix and hyperplasia in fibroblast cells were observed with the examination of collagen fibers (Figure 1b).

During pregnancy, the renin-angiotensin system (RAS) affects fetal growth and development. Ang1, the agonist factor of tyrosine kinase (TEK), has shown[21] that it has anti-inflammatory properties that maintain vascular stabilization of endothelial cells and endothelial permeability in the vascular structure after normal delivery. It has been reported[27,28] that the Ang (1-7)-ACE2-mas receptor axis has an effect on blood pressure regulation during pregnancy, as decreased plasma Ang (1-7) levels are seen in preeclamptic women. Merrill et al[28] showed that nulliparous preeclamptic third trimester patients matched with normal pregnant patients for parity, ethnicity, and gestational age had significantly lower plasma Ang (1-7) concentrations[29]. In our study, an increase in the expression of angiopoietin-1 was observed in the amniotic epithelium, endothelial cells and pericyte cells in the preeclampsia group. This was thought to be due to the effect of the signal induced by trophoblastic invasion. Vimentin is an important extracellular protein content that provides the coordination of the fibroblast and some blood vessels endothelial cells with the amniotic epithelium and the basement membrane of mesenchymal origin. In the control group, angiotensin-1 expression was weakly observed in the amniotic epithelium outside the umbilical cord, blood vessels endothelial

cells, fibroblast and pericyte cells (Figure 1d). It was observed that the expression of Angiotensin-1 increased in the amnion epithelial cells, endothelial cells and some pericyte cells in the HELLP group, and angiogenetic and inflammatory reaction characteristic was induced (Figure 1e).

Epithelial-Mesenchymal conversion (EMT) is an important mechanism for trophoblast invasion and cancer progression[30]. Vimentin is an intermediate fleet protein and is a mesenchymal protein indicating that cell-cell adhesions are lost during EMT. In trophoblast invasion and cancer metastasis, vimentin expression increases[31]. Irtegun et al[32] have shown that the vimentin expression in preeclamptic placements increased by normal placentas and the placental root villus of the vimentin is organized as increased concentric layers around the vessels. They stated that preeclampsia has stimulated the expression of vimentin. It was also seen in our study that vimentin expression increased as a result of severe preeclampsia. Fetal complications in preeclampsia are due to a decreased placental blood flow. Transient changes in vimentin phosphorylation and activation of p21-activated kinase 1 (PAK1), a kinase that regulates vimentin filament assembly, occur due to the hypoxia effect. Vimentin has been shown to increase the permeability of acrylamide endothelium, which disrupts vimentin filamentous network and vimentin filaments. In the control group, vimentin reaction was weak in the basement membrane of the amniotic epithelium, in the intimal layer of the blood vessel, and in the smooth muscle cells (Figure 1e). In the HELLP group umbilical cord section, an increase in vimentin expression was observed in the basal membrane of the amniotic epithelium and the in the fibroblast cells found Wharton gel, in the blood vessels, in the smooth muscle cells in the tunica media layer (Figure 1f).

It has been observed that the change in endothelial permeability causes disturbances in blood flow and thinning of collagen structures as well as changes in smooth muscle circulation. In a study[33] on preeclampsia, it was observed that endothelial cell nuclei showed shallow and deep invaginations, swollen mitochondria, and cristae fragmentation, endoplasmic reticulum was highly enlarged and vacuolated. In the HELLP group, marked hyperplasia in endothelial cells, degenerative changes in mitochondria and loss of cristae, degeneration and thinning of collagen structures in the intimal region were observed[34-38]. In the control group, chromatin intensity was low and endothelial cells were protruding towards the lumen in blood vessels. In addition, mitochondrial structures were regular (Figure 1g). In the HELLP group, a significant hyperplasia in endothelial cells, degenerative changes in mitochondria, degeneration and thinning of collogen structures in the intimal area (Figure 1h) were observed.

Limitations

Patient number could be increased. Western blot could be performed to quantitatively support immunohistochemical staining.

Conclusions

As a result, it was observed that the signaling that started with trophoblastic invasion with the effect of hypoxia in severe preeclampsia and continued with dysfunction in endothelial cells was parallel to the increase in angiotensin and vimentin receptors. It is thought that the ultrastructural change in endothelial cells may cause the disruption of the collagenized structure in Wharton gel, which supports this, and may cause adverse effects in fetal development and nutrition.

Conflict of Interest

All authors have nothing to disclose.

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Ethics Approval

Ethical approval of the study was obtained from Van Akdamar Hospital Non-Interventional Clinical Trials Ethics Committee (decision No.: 2022/01-02).

Informed Consent

All patients were informed about the study and signed the informed patient consent form.

Authors' Contributions

All authors contributed equally to manuscript drafting, writing, data collection, conceptualization and observation.

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