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MOLECULAR-PATHOGENETIC ASPECTS OF HIF-1α PARTICIPATION IN THE DEVELOPMENT OF INTRANATAL FETAL DEATH IN PREECLAMPSIA

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Abstract
This study investigates the role of hypoxia-inducible factor 1-alpha (HIF-1α) in the molecular pathogenesis of intranatal fetal death complicating preeclampsia. Using immunohistochemistry, Western blotting, and ELISA analysis of placental tissue and serum samples from 65 women, we demonstrate significantly elevated HIF-1α expression in preeclamptic placentas, correlating with sFlt-1 upregulation, PlGF suppression, and fatal perinatal outcomes.

Keywords: HIF-1α, preeclampsia, intranatal fetal death, hypoxia-inducible factor, placental hypoxia, sFlt-1, PlGF, trophoblast dysfunction, immunohistochemistry, Western blot, angiogenic imbalance, molecular pathogenesis, oxidative stress, fetoplacental insufficiency, perinatology.

Introduction

Preeclampsia affects 3-8% of all pregnancies worldwide and remains one of the leading causes of maternal and perinatal mortality, responsible for approximately 76,000 maternal and 500,000 perinatal deaths annually according to WHO 2022 data. Intranatal fetal death - defined as fetal demise occurring during the active course of labor - represents the most severe perinatal consequence of preeclampsia, with an incidence of 1.2-2.8 per 1,000 deliveries in affected pregnancies. Despite decades of clinical research, the molecular mechanisms linking placental hypoxia, aberrant angiogenic signaling, and acute intrapartum fetal compromise remain incompletely characterized. Hypoxia-inducible factor 1-alpha (HIF-1α) - the master transcriptional regulator of cellular hypoxic response - has emerged as a central molecular mediator of placental dysfunction in preeclampsia. Understanding HIF-1α-driven pathways at the tissue and protein level may provide the mechanistic basis for developing early biomarker strategies to identify pregnancies at highest risk of intranatal catastrophe.

Literature review

I.S. Sidorova and N.A. Nikitina (2011) established the central role of uteroplacental ischemia in preeclampsia pathogenesis and described hypoxia-driven angiogenic factor dysregulation as a defining molecular feature. E.K. Aylamazyan and M.S. Zainulina (2010) analyzed oxidative stress and endothelial dysfunction in severe preeclampsia, identifying reactive oxygen species as co-activators of HIF-1α stabilization. A.D. Makatsariya et al. (2013) demonstrated the interaction between thrombophilic states and placental hypoxic signaling, linking HIF-1α activation to spiral

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artery thrombosis. M.A. Repina (2010) examined eclampsia and severe preeclampsia from a systemic pathophysiology perspective, correlating clinical severity with placental molecular changes. T.U. Kuzminykh (2014) specifically measured hypoxia marker expression including HIF-1α in preeclamptic placental tissue using immunohistochemical methods. Uzbek researchers G.N. Xasanova and F.R. Tursunova (2019) described molecular markers of fetoplacental insufficiency in severe preeclampsia in the regional obstetric population. V.N. Serov and G.T. Sukhikh (2015) reviewed current molecular pathogenesis concepts of preeclampsia, placing HIF-1α within the broader antiangiogenic cascade. These works form the scientific foundation of the present investigation.

Methodology

This prospective case-control morphological and biochemical study enrolled 65 women who delivered at a tertiary perinatal center. Three study groups were formed: Group I - 25 women with severe preeclampsia complicated by intranatal fetal death (SIFD group); Group II - 25 women with severe preeclampsia and live birth (SPE group); Group III - 15 healthy women with uncomplicated singleton pregnancies and live birth (control group). Diagnosis of severe preeclampsia was established according to WHO 2011 and national clinical protocol criteria: systolic blood pressure ≥160 mmHg, diastolic ≥110 mmHg on two measurements 4 hours apart, proteinuria ≥5.0 g/24 hours, and at least one severe feature (thrombocytopenia, renal insufficiency, hepatic dysfunction, or neurological symptoms). Intranatal fetal death was defined as confirmed absence of fetal cardiac activity during labor after 28 weeks of gestation with no antepartum registration of fetal demise. Exclusion criteria for all groups: chromosomal fetal anomalies, multiple gestation, pre-existing diabetes mellitus, documented thrombophilia, and chronic hypertension diagnosed before 20 weeks. Mean gestational age at delivery: Group I - 34.6 ± 2.1 weeks; Group II - 35.8 ± 1.9 weeks; Group III - 38.7 ± 0.8 weeks. Mean systolic blood pressure at admission: Group I - 178.4 ± 9.3 mmHg; Group II - 169.7 ± 8.1 mmHg; Group III - 118.6 ± 6.4 mmHg. Mean proteinuria: Group I - 6.8 ± 1.4 g/24h; Group II - 5.4 ± 1.1 g/24h; Group III - 0.08 ± 0.02 g/24h. Mean platelet count at admission: Group I - 98.3 ± 14.7 × 10^9/L; Group II - 124.6 ± 18.3 × 10^9/L; Group III - 234.7 ± 22.1 × 10^9/L.

Placental specimens were obtained immediately following delivery. Full-thickness samples (1.0 × 1.0 × 0.5 cm) were excised from four standardized zones: central, paracentral, peripheral, and umbilical cord insertion site. For immunohistochemistry and Western blot: tissue fixed in 10% neutral buffered formalin for 24 hours, dehydrated through graded alcohols, cleared in xylene, and paraffin-embedded; serial sections of 4-5 μm cut on Leica RM2235 microtome. For ELISA: tissue samples snap-frozen in liquid nitrogen and stored at −80°C until protein extraction. Maternal peripheral blood (10 mL) drawn at admission for serum sFlt-1, PlGF, and VEGF quantification.

Paraffin sections were deparaffinized in xylene and rehydrated through descending alcohols. Antigen retrieval was performed in 10 mM citrate buffer (pH 6.0) at 95°C for 20 minutes. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 minutes. Sections were incubated overnight at 4°C with primary anti-HIF-1α rabbit monoclonal antibody (Abcam ab51608, 1:200 dilution). Secondary biotinylated anti-rabbit antibody applied for 30 minutes at room temperature, followed by streptavidin-HRP complex. Visualization performed with DAB chromogen;

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counterstaining with Mayer's hematoxylin. HIF-1α expression was quantified using H-score: H-score = Σ(staining intensity × percentage of positive cells), range 0-300.

Placental tissue (50 mg) was homogenized in RIPA lysis buffer with protease inhibitor cocktail. Protein concentration determined by BCA assay. Denatured proteins (30 µg per lane) separated on 10% SDS-PAGE gel and transferred to PVDF membrane. Membranes blocked with 5% non-fat dry milk in TBST for 1 hour. Primary anti-HIF-1α antibody (1:1000) incubated overnight at 4°C; secondary HRP-conjugated antibody (1:5000) for 1 hour. Detection by enhanced chemiluminescence (ECL). Band intensity normalized to β-actin as loading control; quantified by ImageJ densitometry.

Serum concentrations of sFlt-1, PlGF, and VEGF-A measured using commercially validated ELISA kits (Quantikine, R&D Systems) per manufacturer's protocol. All samples analyzed in duplicate; intra-assay coefficient of variation <8%. Data expressed as mean ± SD or median (IQR). Between-group comparisons performed using Kruskal-Wallis test with post-hoc Dunn's correction. Spearman correlation used for relationship between HIF-1α H-score and serum angiogenic factors. Statistical significance threshold: $p<0.05$. Analysis performed in SPSS Statistics 26.0 and GraphPad Prism 9.0.

Results

In the control group, HIF-1α immunoreactivity was low and confined primarily to syncytiotrophoblast cytoplasm, with a mean H-score of 58.4 ± 9.2. In the SPE group (severe preeclampsia, live birth), HIF-1α expression was significantly elevated, with a mean H-score of 164.7 ± 18.3 ($p<0.001$ vs. control), with strong nuclear and cytoplasmic staining in syncytiotrophoblast, cytotrophoblast, and villous stromal cells. In the SIFD group (intranatal fetal death), HIF-1α H-score reached 231.8 ± 22.6 - significantly higher than both the SPE group ($p=0.002$) and controls ($p<0.001$). Nuclear translocation of HIF-1α, indicating transcriptional activation, was observed in 91.4% (n=32/35 evaluated fields) of SIFD specimens versus 61.2% in SPE and 8.3% in controls. The proportion of HIF-1α-positive trophoblast cells per ×400 field measured 74.3 ± 8.1% in SIFD, 51.6 ± 7.4% in SPE, and 18.2 ± 4.3% in controls ($p<0.001$ for all pairwise comparisons).

Western blot confirmed IHC findings with objective protein-level quantification. HIF-1α/β-actin band density ratio in the SIFD group was 3.84 ± 0.41, representing a 3.8-fold increase over controls (1.00 ± 0.12, set as reference). The SPE group showed an intermediate elevation of 2.43 ± 0.29 (2.4-fold over control; $p<0.001$ vs. control, $p=0.003$ vs. SIFD). These differences were statistically significant across all three pairwise comparisons ($p<0.001$).

Serum sFlt-1 concentrations were markedly elevated in preeclamptic groups: SIFD group - 14,382 ± 1,847 pg/mL; SPE group - 9,614 ± 1,203 pg/mL; control group - 1,842 ± 294 pg/mL ($p<0.001$, SIFD vs. control; $p=0.004$, SIFD vs. SPE). Serum PlGF showed inverse pattern: SIFD - 18.4 ± 4.7 pg/mL; SPE - 47.3 ± 9.1 pg/mL; control - 214.6 ± 31.3 pg/mL ($p<0.001$ for all comparisons). The sFlt-1/PlGF ratio in the SIFD group reached 781.6 ± 143.2 versus 203.2 ± 41.7 in SPE and 8.6 ± 1.4 in controls ($p<0.001$). Serum VEGF-A was paradoxically reduced in both preeclamptic groups despite HIF-1α elevation: SIFD - 43.2 ± 8.6 pg/mL; SPE - 89.4 ± 14.3 pg/mL; control - 187.3 ± 22.7 pg/mL ($p<0.001$).

Spearman correlation analysis revealed a strong positive correlation between placental HIF-1α H-score and serum sFlt-1 ($r=0.81$, $p<0.001$) and a strong inverse correlation between HIF-1α H-score and serum PlGF ($r=-0.79$, $p<0.001$). HIF-1α H-score also correlated positively with proteinuria level

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(r=0.73, p<0.001) and inversely with umbilical artery diastolic flow velocity (r=−0.68, p<0.001), establishing HIF-1α overexpression as a converging molecular node between placental hypoxia, angiogenic imbalance, and deteriorating fetal hemodynamics.

Discussion

The central finding of this study - that placental HIF-1α expression is not merely elevated in severe preeclampsia but reaches its highest levels in cases culminating in intranatal fetal death - provides molecular-level evidence for a hypoxia-driven cascade that ultimately overwhelms fetal circulatory compensatory capacity during labor. HIF-1α is a transcription factor that under normoxic conditions is rapidly ubiquitinated by the von Hippel-Lindau protein and degraded by the proteasome. Under hypoxia, prolyl hydroxylation is inhibited, HIF-1α accumulates, translocates to the nucleus, and drives transcription of dozens of target genes. In the preeclamptic placenta, defective trophoblast invasion of spiral arteries - which normally become wide, low-resistance conduits - results in persistently narrow, high-resistance vessels that create chronic intermittent ischemia in the intervillous space. This is the primary driver of HIF-1α stabilization. Our finding of nuclear HIF-1α translocation in 91.4% of SIFD specimens directly confirms that active HIF-1α transcriptional programs were operating in the terminal placental compartment of these pregnancies.

The paradox of elevated HIF-1α with reduced rather than elevated VEGF-A deserves explanation. Under acute and moderate hypoxia, HIF-1α increases VEGF-A transcription - a pro-angiogenic response. However, in the chronically hypoxic preeclamptic placenta, sFlt-1 - a soluble decoy receptor for VEGF that is itself transcriptionally upregulated by HIF-1α - is produced in massive excess, binding and neutralizing free VEGF-A in both placental tissue and maternal serum. The sFlt-1/PlGF ratio of 781.6 in the SIFD group confirms an extreme antiangiogenic state. Free VEGF-A is effectively quenched, endothelial maintenance is lost, and placental vascular integrity deteriorates. This mechanism, described at the population level by Sidorova and Nikitina (2011) and at the molecular level by Kuzminykh (2014), here receives quantitative confirmation in the subset of cases where this cascade reached its most devastating clinical endpoint. The strong correlation between HIF-1α H-score and umbilical artery diastolic flow velocity (r=−0.68) is particularly instructive. As HIF-1α-driven sFlt-1 excess progressively damages placental villous capillary endothelium, fetal vascular resistance in the placenta rises - eventually leading to absent or reversed end-diastolic flow, a recognized terminal finding in intrapartum fetal compromise. The molecular data from this study thus provide a mechanistic explanation for a clinical Doppler finding that is widely used but whose underlying biology was previously inferred rather than directly demonstrated. The significantly higher HIF-1α expression in SIFD compared to SPE with live birth (H-score 231.8 vs. 164.7; protein ratio 3.84 vs. 2.43) suggests a quantitative threshold effect: beyond a certain level of HIF-1α-driven angiogenic and vascular injury, the fetus loses its ability to compensate during the additional hemodynamic stress of active labor contractions. This has direct clinical implications - HIF-1α or sFlt-1/PlGF ratio measured at hospital admission for labor could serve as a rapid molecular stratification tool, identifying fetuses requiring immediate delivery rather than a trial of labor.

Placental HIF-1α overexpression constitutes a central molecular mechanism in the pathogenesis of intranatal fetal death complicating preeclampsia, driving sFlt-1-mediated angiogenic collapse, villous vascular deterioration, and progressive fetal hemodynamic failure. The quantitative gradient of HIF-

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1α expression between severe preeclampsia with live birth and intranatal fetal death identifies this factor as a biologically meaningful severity stratifier with potential clinical utility in intrapartum risk assessment.

References:

1. Сидорова И.С., Никитина Н.А. Преэклампсия в центре внимания практикующего акушера // Акушерство и гинекология. - 2011. - № 4. - С. 12-19.
2. Айламазян Э.К., Зайнулина М.С. Эндотелиальная дисфункция и оксидативный стресс при тяжёлой преэклампсии // Журнал акушерства и женских болезней. - 2010. - № 2. - С. 3-10.
3. Макацария А.Д., Бицадзе В.О., Акиньшина С.В. Тромбофилия и антитромботическая терапия в акушерской практике. - М.: Триада-Х, 2013. - 904 с.
4. Репина М.А. Эклампсия: ошибки акушерской тактики // Журнал акушерства и женских болезней. - 2010. - № 3. - С. 18-26.
5. Кузьминых Т.У. Молекулярные маркёры гипоксии плаценты при тяжёлой преэклампсии // Акушерство и гинекология. - 2014. - № 6. - С. 34-39.
6. Хасанова Г.Н., Турсунова Ф.Р. Оғир преэклампсияда фетоплацентар етишмовчиликнинг молекуляр маркёрлари // Ўзбекистон акушерлик-гинекология журнали. - 2019. - № 1. - Б. 31-37.
7. Серов В.Н., Сухих Г.Т. Клинические рекомендации. Акушерство и гинекология. - 4-е изд. - М.: ГЭОТАР-Медиа, 2015. - 1024 с.

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