Participation of hypoxia-inducible factor-1α in the pathogenesis of preeclampsia-related placental ischemia and its potential as a marker for preeclampsia

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Abstract
Hypoxia-inducible factor-1α (HIF-1α) is important for placental development. This study aims to determine whether the increased level and expression of HIF could be used to demonstrate failed placentation in women with preeclampsia. The study included 20 pregnant females [10 with and 10 without preeclampsia (the control group)]. Placental tissues were collected and stained with hematoxylin and eosin. Immunohistochemical studies for evaluating the expression of HIF-1α by these tissues were then performed. The results demonstrated that placental tissues collected from mothers with preeclampsia showed a variety of histomorphological changes. All the cytotrophoblasts rimming the placental villi in mothers with preeclampsia demonstrated a strong and uniform nuclear staining with HIF-1α. The study results indicated that cytotrophoblasts respond to an ischemic environment by their nuclear expression of HIF-1α, and thus we conclude that this transcription factor has a significant potential as a marker for preeclampsia.

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Introduction

Preeclampsia is a pregnancy-specific disorder. At present, the definition of this disorder is solely based on physical signs such as hypertension and proteinuria,\(^1\)
\(^2\) and its etiology needs further investigation. Therefore, preeclampsia is defined as pregnancy-induced hypertension and proteinuria. According to the American College of Obstetricians and Gynecologists guidelines, preeclampsia occurs in 3–5% of pregnant women worldwide and normally manifests during the 20th week of gestation.\(^3\)

Placentation is a process of molecular mimicry or pseudo-vasculogenesis, in which the trophoblasts acquire an endovascular phenotype and invade the uteroplacental spiral arteries.\(^4\) The arteries, which are initially elastic and of small caliber, are converted into inelastic and dilated tubes, devoid of maternal vasomotor control. This physiological remodeling provides optimal perfusion and oxygen supply for maintenance of pregnancy. More specifically, the uteroplacental arteries lose their musculature and enlarge up to four to six times the diameter of the same vessel in nonpregnant women.\(^5\)
\(^6\)

The process of placentation is known to be aberrant in women with preeclampsia. In this case, the cytotrophoblasts fail to adopt the invasive endothelial phenotype that is necessary for normal physiological remodeling.\(^7\)\(^8\) Therefore, the uteroplacental spiral arteries remain only as small-caliber elastic vessels. This is failed, abnormal, or aberrant pseudovasculogenesis. As a result, there is reduced blood flow to the placenta, which results in placental ischemia or hypoxic placenta. A hypoxic placenta then releases numerous vasoactive factors such as tumor necrosis factor-1, tumor necrosis factor 1-\(\alpha\), and interleukin-6 that cause a decrease in nitric oxide bioavailability and an increase in reactive oxygen species. A combination of these two events results in an increase in total peripheral resistance and manifests as hypertension.\(^9\)

A hypoxic placenta causes accumulation of placenta-generated soluble factors. There can be a mixed consequence to this depending on the effects of the factors produced. Potent angiogenic factors that play an important role in the stimulation of blood vessel growth, such as vascular endothelial growth factor (VEGF), soluble fms-like tyrosine kinase 1 (sFlt1), and placental growth factor have been suggested to be present in preeclamptic placentae.\(^10\) Another placenta-soluble substance released is hypoxia-inducible factor \(1\alpha\) (HIF-1\(\alpha\)), and its effects, expression, and regulation have been evaluated previously.\(^11\)\(^12\)

HIF consists of two members of the basic helix–loop–helix transcription factor family, namely, HIF-1\(\alpha\) and HIF-1\(\beta\). The HIF-1\(\alpha\) is a short-lived protein and is the transcription factor for a large number of genes. It is known to mediate the delivery of oxygen and other nutrients through the induction of angiogenesis.\(^13\)\(^14\) HIF-1\(\alpha\) binds to hypoxia-response elements in promoting the activity of many genes involved in the adaptation to an environment of insufficient oxygen supply (hypoxia). It is a major regulator of cell function and differentiation, and mediates the effects of oxygen on human trophoblast development.\(^12\)\(^14\) On the downside, however, HIF-1\(\alpha\) is known to activate the transcription factor of VEGF and promotes tumor cells to survive under hypoxic conditions, as VEGF is a key factor in tumor angiogenesis.\(^13\)\(^–\)\(^15\)

Results of previous studies have demonstrated the presence of HIF-1\(\alpha\) in preeclamptic placentae.\(^16\) HIF-1\(\alpha\) has also been shown to be strongly expressed from the 5th week of gestation onward, especially in the syncytiotrophoblast, villous cytotrophoblast, and extravillous trophoblast.\(^17\) The increase in the levels of HIF-1\(\alpha\) is closely related to the increase in the levels of another protein called telomerase or hTERT. Further studies have identified that the placental production of HIF-1\(\alpha\) mediates the increase in the levels of telomerase during hypoxia, which plays a role in the modulation and proliferation of trophoblasts.\(^14\)\(^15\) However, whether the increase in levels of this protein occurs in parallel with severe aberrant pseudovasculogenesis requires further investigation.

This pilot study aimed to identify the histological and immunohistochemical differences in placental pseudo-vasculogenesis by performing a biopsy on the placentae of mothers with and without preeclampsia. It also aimed to demonstrate staining patterns of HIF-1\(\alpha\) and investigated whether the transcription factor HIF-1\(\alpha\) can function as a marker for preeclampsia.

Materials and methods

This is a case–control pilot study. All of the 20 pregnant mothers in this study provided their consent for participation and their placental tissue was harvested. Feasibility of this study was discussed with the Department of Obstetrics and Gynaecology (Ampang Hospital, Kuala Lumpur, Malaysia). Mothers were identified and recruited by co-investigators from the Ampang Hospital. Patients’ particulars and clinical data including their name, age, date of admission, para, gravida, blood group, blood pressure, and urine protein levels were recorded subsequent to obtaining informed and written consent.

This study was conducted according to the guidelines stated in the Declaration of Helsinki. The study protocol was approved by the Institutional Ethics Committee.

Inclusion criteria were as follows: mothers who were newly diagnosed cases of preeclampsia and who were not more than 45 years of age, pregnant mothers with systolic blood pressure more than 140 mmHg and diastolic blood pressure more than 90 mm Hg, and 24-hour urine protein levels in the nephrotic range at diagnosis. Only consenting mothers were recruited for the study.

Exclusion criteria were patients with other comorbidities such as diabetes mellitus and essential hypertension in an effort to preclude morphological effects of these conditions on blood vessels inclusive of uteroplacental spiral arterioles. Nonconsenting patients were excluded from the study.

Because this is a preliminary study, the sample size was limited to 20 cases, and a biopsy of the placentae of all participants was performed. The patients were divided into two experimental groups, namely, Group 1 and Group 2. Experimental Group 1 consisted of 10 pregnant mothers with newly diagnosed preeclampsia without comorbidities. Experimental Group 2 consisted of 10 pregnant mothers without comorbidities.
Placental tissue was collected from mothers delivering vaginally as well as by cesarean section. The technique of grossing of the placental tissue was restricted to 4-mm-thick, flat, shaved sections of the basal plate of the placenta. The tissues were embedded en face in anticipation of demonstration of increased numbers of spiral arterioles as compared with traditional full-thickness longitudinal sections of placenta. It was presumed that using en face shaved sections of the placental base exposed more surface area of the bed in proximity to the opposing myometrium, indirectly increasing a view of the feto-maternal interface blood vessels. Randomly shaved en face sections (approximately 4-mm thick) of the basal plate of the placenta were taken, sealed into labeled cassettes (histosettes), and fixed in formalin for about 3 weeks prior to processing. Sections were stained with hematoxylin and eosin and immunohistochemical marker HIF-1α. The placental sections were studied to document and grade features of placental ischemia in mothers with pre-eclampsia. Spiral arterioles were identified and patterns of staining were documented and analyzed. The immunohistochemical staining patterns were noted and the staining intensity (from 1+ to 3+) and location were identified. The histology study results of the placenta from normal mothers were used for comparison.

Results

Mothers with preeclampsia were aged between 20 years and 35 years with uterine fundal heights ranging between 29 weeks and 34 weeks. The placental weights in normal mothers were greater when compared with the pre-eclamptic mothers. Shaved sections of the placental floor demonstrated an average of four spiral blood vessels per case.

Placental tissues from normal pregnant mothers demonstrated the presence of a uniform, small population of well-vascularized third trimester placental villi collared by cytotrophoblasts. Syncytiotrophoblasts were noted to be normal in number and their distribution and syncytial knots were not significantly increased. Placental floor infarcts were not recognized and fibrin intervals were scanty. Atherosis was not demonstrated (Fig. 1A). However, fractured amorphous calcifications were evident in a few cases consistent with an aging placenta.

Placenta from mothers with preeclampsia showed a variety of histomorphological changes. The placental villi were of varying sizes (geographic villi) and exhibited significantly increased numbers of syncytial knots. Several sclerotic villi were noted. Significant amounts of inter-villous fibrin were seen to entrap sclerotic placental villi. Chorangiosis was a consistent feature of ischemic changes in placentae of mothers with preeclampsia (Fig. 1B). Atherosis was not a consistent feature of all placentae from mothers with preeclampsia, whereas infarcts of the placental floor were a consistent feature. Chunky, amorphous, fractured calcifications were also noted.

The histomorphological features of placental ischemia were corroborated with the staining patterns of immunohistochemical marker. The HIF-1α immunohistochemical staining was performed on both normal and preeclamptic placentae. It was found that the cytotrophoblasts rimming the placental villi in normal mothers did not reflect a nuclear uptake of the HIF-1α stain. By contrast, all the cytotrophoblasts rimming the placental villi in mothers with preeclampsia demonstrated a strong and uniform nuclear staining with HIF-1α. Positive staining was not appreciated in the cells lining the spiral arterioles in a significant manner (Fig. 2).

Discussion

This study evaluated the potential of HIF-1α as a predictive marker for preeclampsia bearing in mind its expression and morphological effects in ischemic environments and in other neoplastic scenarios. The role of HIF-1α in the pathogenesis of preeclampsia and whether this transcription factor can function as a marker for preeclampsia are still being investigated. In preeclampsia, the transcription factor HIF-1α is reported to be best characterized in placental trophoblasts,13,17 which was also consistently evident in our study.

The use of 4-mm shaved en face sections of the placental base afforded a greater yield of spiral arterioles as compared with traditional longitudinal sections, because the latter did not reflect as much surface area of the

Figure 1  (A) A normal third trimester placenta. Uniform, small, and well-vascularized placental villi rimmed by cytotrophoblasts and syncytiotrophoblasts. The syncytiotrophoblasts are normal; no atherosis is noted. (B) Decidua showing the cross section of three spiral arterioles. The walls display a significant degree of fibrinoid necrosis indicative of ongoing atherosis (hematoxylin and eosin, 200×).
feto-maternal interface. However, this study does not conclude with confidence that the shaved sections can replace myometrial biopsies (placental bed). The confirmation of placental ischemia by histomorphological and immunohistochemical studies was successful to a large extent due to the use of shaved en face sections of the placental base that gave a significantly greater yield of spiral arterioles. This finding correlated with the observations of other authors who suggested that shaved sections of the placental base were equivalent to bed biopsies in the assessment of arteriole morphology. A dedicated study to compare spiral arteriole populations demonstrable in both types of biopsy could be one of the future directions that could aid in the better understanding of feto-maternal vasculature and its related problems, whether anatomical or thrombogenic.

Atherosis, in addition to fibrinoid degeneration of blood vessel walls, also includes the presence of foamy/clear cells within the vascular lumina. The propensity of the HIF-1α to duplicate the morphological evidence of producing clear cells in several scenarios propelled the hypothesis that HIF-1α is likely to be expressed by cytotrophoblasts in a hypoxic environment. During its presence in ischemic environments, it is expressed as a transcription factor and additionally leaves the evidence of its existence in the form of clear cells (atherosis). Elevated levels and expression of HIF-1α in mothers with preeclampsia did not impact the placental weights directly, which can be interpreted as the mothers with the highest levels of the transcription factor did not exhibit the lowest placental weights. Again, this finding suggests that the transcription factor reflects an ischemic environment and does not contribute to the pathogenesis of the consequences of aberrant pseudovasculogenesis such as reduced placental weights and intrauterine growth restriction.

Atherosis, aberrant pseudovasculogenesis and the subsequent placental ischemia is a consistent feature of placentae of mothers manifesting with preeclampsia. As discussed by other authors, infarction and syncytial knots were a common feature in placenta of mothers with preeclampsia, and this was duplicated in all cases of preeclampsia in this study. The other consistent feature indicative of placental ischemia was increased syncytial knots. The manifestation of geographic placental villi was also described by some authors, as accelerated maturation was again noted to be a consistent feature of ischemia in placentae of mothers with preeclampsia.

The expression of HIF-1α by the nuclei of all cytotrophoblastic cells in the placental villi of mothers with preeclampsia suggests that there is an overexpression of HIF-1α in hypoxic placentae. This finding was supported by the histologic correlates of placental ischemia in the present study and the increased levels of HIF-1α in prenatal blood samples of mothers with preeclampsia. The expression of HIF-1α by cytotrophoblasts and its association with elevated blood levels of HIF-1α in mothers with preeclampsia suggest that the cytotrophoblasts reflect and contribute to the ischemic environment. HIF-1α distribution was uniform within all cytotrophoblastic cells with a strong nuclear accentuation, which suggests a consistent expression of this transcription factor within these cells in preeclampsia. It could be an indirect indicator of absence of blood vessel wall invasion (aberrant pseudovasculogenesis) creating an ischemic environment that leads to the expression of HIF-1α within cytotrophoblastic cell nuclei.

The identification of HIF-1α within the cytotrophoblastic cell nuclei confirms the expression of this transcription factor within cells that are stipulated to play the most important role in instituting successful placentation but have failed to achieve this in preeclampsia. This indicates that cytotrophoblasts respond to and reflect an ischemic environment by their nuclear expression of HIF-1α, which suggests that this transcription factor has great potential to be further investigated as a blood marker for preeclampsia.

A previous study suggested that pre- and postnatal blood samples of normal mothers showed baseline levels of the transcription factor. Although this does suggest that pregnancy as a physiological stressful condition could by itself induce a minor degree of ischemia in all mothers, it could also suggest that the future direction of trying to establish the credibility of HIF-1α as a predictive marker in preeclampsia requires the measurement of this transcription factor in nonpregnant women of similar age groups.

In conclusion, HIF-1α shows great potential of being a consistent and reliable predictive blood marker for preeclampsia. It opens up greater opportunities for further research on the topic across races and population groups.
Conflicts of interest

The authors declare that they have no conflicts of interest in this study.

References